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THE COMPLEX NATURE OF WHITE-CLOVER MOSAIC

FOLKE JOHNSON[1]

(Accepted for publication May 26, 1941)

INTRODUCTION

White-clover mosaic, a disease that causes streak when transmitted to the garden pea (*Pisum sativum* L.) (13, 14, 24, 25), has been regarded as induced by a single virus, classified by Weiss (18) as Trifolium virus 1. In a recent study (7) on the transmission of viruses by dodder (*Cuscuta campestris* Yunck.), the writer isolated two distinct entities from white clover plants (*Trifolium repens* L.) affected by mosaic. Separation of the two viruses was possible because dodder transmitted only one of them, and the cowpea (*Vigna sinensis* (L.) Endl.) was susceptible only to the other. The virus transmitted by dodder will be referred to as pea-mottle virus and that isolated by means of cowpea will be designated as pea-wilt virus. The present paper presents the results of a study of the properties of these two viruses, their characteristic reactions, and their probable relationships with other legume viruses.

LITERATURE REVIEW

The literature reveals the fact that symptoms of streak in pea may be caused by any one of a number of distinct viruses or virus complexes. Linford (9) noted that pea streak was present in fields from the Atlantic coast to Utah and Montana. He subsequently showed (10) that in Hawaii a similar disease was produced in peas by the virus of pineapple yellow spot, now known to be identical (12, 15) with tomato spotted-wilt virus (*Lethum australiense* H.).[2] Linford's results were confirmed by Whipple (19) and Snyder and Thomas (16), who reported that spotted-wilt virus caused streak in garden peas and sweet peas. Adam (1), in South Australia, obtained results identical with those of Whipple. In a recent communication, Whipple and Walker (20) described two viruses believed to be strains of the common cucumber-mosaic virus (*Marmor cucumeris* H.), both of which caused streak in certain field-grown peas in Wisconsin. Further evidence that strains of the cucumber-mosaic virus were widespread in nature and caused streak in garden peas was given by Zaumeyer (23). Stubbs (17) reported that tobacco-ringspot virus (*Annulus tabaci* H.) caused streak and death of inoculated pea plants. Zaumeyer (22) described an outbreak of streak in garden peas exposed to pea aphids (*Macrosiphum pisi* Kaltenbach) collected from field-grown alfalfa (*Medicago sativa* L.) and believed the disease to be caused by a distinct virus, which he called pea-streak virus 1; Zaumeyer also isolated two strains of alfalfa-mosaic virus (*Marmor medi-*

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[2] The Latin names used in this paper follow the system of nomenclature presented in the Handbook of Phytopathogenic Viruses (5).

caginis H.), which gave symptoms nearly comparable to streak when used to infect peas. A systemic streak in greenhouse-grown peas inoculated with juice from mosaic-infected white clover was observed by Zaumeyer and Wade (24, 26) and by Pierce (13, 14); while Osborn (11) noted a similar disease caused by the red clover vein-mosaic virus (*M. trifolii* H.). Chamberlain (4) reported a new virus that caused pea streak in New Zealand, and more recently Ainsworth and Ogilvie (2, 3), showed that lettuce-mosaic virus (*M. lactucae* H.) caused streak and death of sweet peas in Great Britain. A single mention of pea streak occurring in China was made by Yu (21). Thus it is known that in addition to the reports of pea streak made by Chamberlain (4) and Yu (21), the viruses of tomato spotted-wilt (pineapple yellow-spot), cucumber mosaic, pea streak, alfalfa mosaic, white-clover mosaic, red-clover vein mosaic and lettuce mosaic caused streak and death to garden peas and sweet peas in various parts of the world.

SEPARATION OF THE VIRUSES FROM THE COMPLEX

Pea-mottle virus was isolated by the following method: 12 white-clover plants infected with the virus complex (Trifolium virus 1) were joined by means of dodder to 12 healthy broad-bean plants (*Vicia faba* L.). Similarly, 8 hop-clover plants (*Medicago lupulina* L.), infected with the virus complex, were connected to 8 healthy hop-clover plants. Nine broad-bean and 5 hop-clover plants became diseased with mosaic during a period of time between 32 and 40 days after connecting them with the diseased plants. The hop-clover plants were mildly mottled and less stunted than those to which they were connected (Fig. 1, *A*). Inoculation of Dwarf Telephone peas with juice from the diseased broad beans and mildly affected hop clover produced symptoms of mosaic (Fig. 1, *B*, b, c) rather than those of streak. The same result was obtained when pea plants were inoculated with juice extracted from dodder that had parasitized infected white clover. On the other hand, inoculations of similar plants with juice from the diseased white clover invariably resulted in streak. The results led to the belief that the white clover was infected with more than one virus, and that dodder had isolated but one constituent of the complex, namely pea-mottle virus.

The second constituent of the complex, referred to as pea-wilt virus, was isolated by inoculation of cowpea with juice from the diseased white clover, since it was found that cowpea plants were not susceptible to infection with pea-mottle virus. Cowpea leaves inoculated with plant juice containing the virus complex developed brown, necrotic, local lesions (Fig. 1, *B*, a). On transfer from infected cowpeas to Hundredfold peas, the virus produced no definite local lesions, but caused wilting and death of the inoculated leaves (Fig. 1, *C*, a). The infected pea plants did not develop streak or chlorotic mottling, but showed only a mild discoloration of the stem; nevertheless, it was easy to demonstrate the presence of virus in the tops of infected plants by inoculation of expressed juice to cowpeas. The pea-wilt virus isolated in this manner, when mixed with the previously isolated pea-

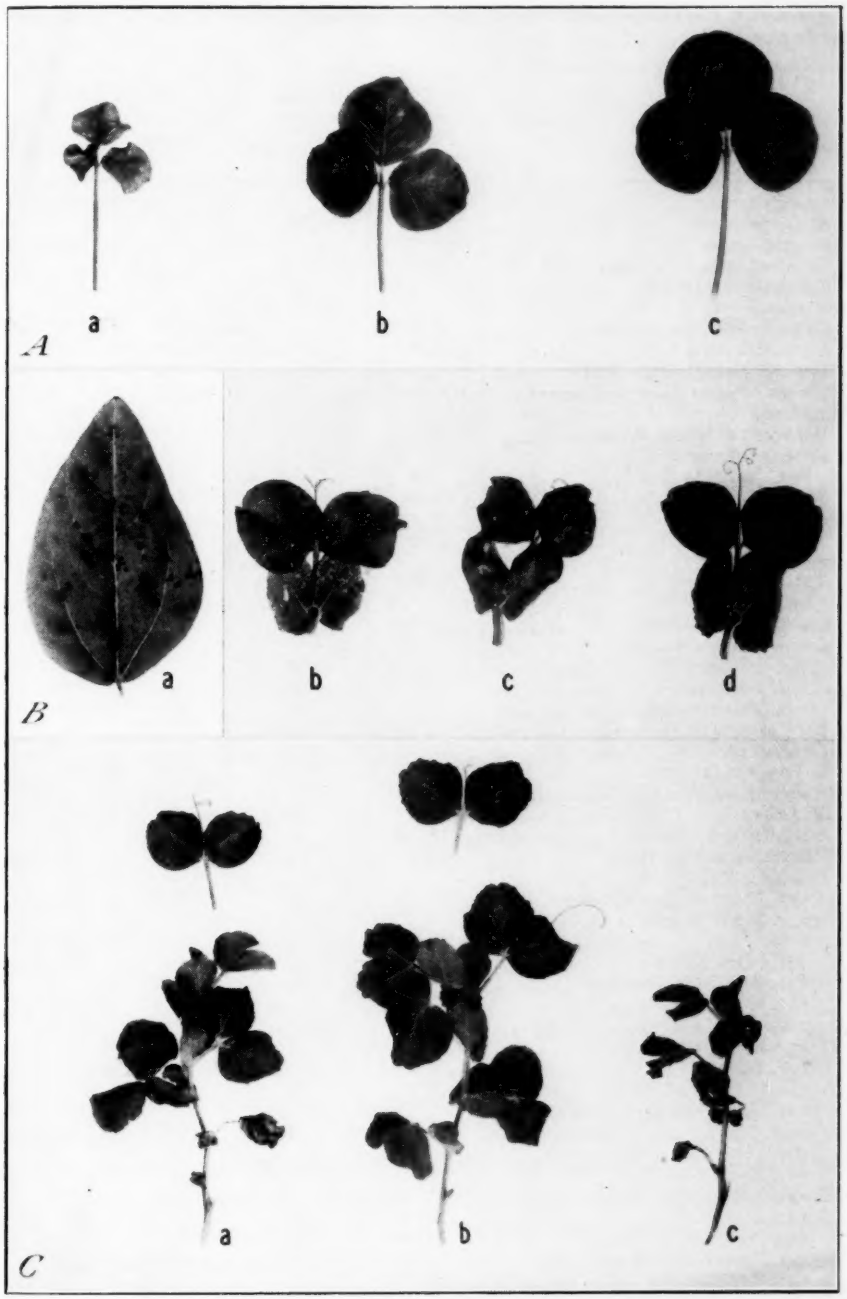

FIG. 1. Symptoms produced by virus complex and separated virus constituents in several plants. *A*, a. Dwarfing and malformation in hop clover caused by virus complex (Trifolium virus 1) from mosaic-infected white clover. b. Mild mottling in hop clover produced by pea-mottle virus. c. Healthy hop-clover leaf. *B*, a. Local necrotic lesions in cowpea produced by pea-wilt virus. b, c. Mosaic in Dwarf Telephone pea caused by pea-mottle virus. Early symptoms at b, later symptoms at c. d. Healthy Dwarf Telephone pea leaves. *C*, a. Death of inoculated leaf resulting in Dwarf Telephone pea from infection of pea-wilt virus. b. Healthy Dwarf Telephone pea plant. c. Streak in Dwarf Telephone pea produced by infection with a combination of pea-mottle virus and pea-wilt virus. (Photographs by Julian A. Carlile.)

TABLE 1.—*Comparative suscept range of pea-mottle virus and pea-wilt virus according to families*

Host plant	Pea-mottle virus		Pea-wilt virus	
	Plants tested[a]	Symptoms[b]	Plants tested	Symptoms
Caryophyllaceae				
Stellaria media (L.) Cyrill.	8/8	M	8/0	
Chenopodiaceae				
Beta vulgaris L. (Sugar beet)	10/0		10/0	
Spinacia oleracea L.	5/5	M	5/0	
Compositae				
Callistephus chinensis Nees	10/0		10/0	
Lactuca sativa L.	10/0		10/0	
Taraxacum officinale Weber	1/0		1/0	
Zinnia elegans Jacq.	20/0		22/0	
Cruciferae				
Barbarea vulgaris R. Br.	1/0		1/0	
Brassica oleracea L.				
var. *capitata* DC.	5/0		5/0	
Raphanus sativus L.	5/0		5/0	
Cucurbitaceae				
Cucumis sativus L.	25/24	YL	25/0	
Gramineae				
Zea mays L.	16/0		19/0	
Leguminosae				
Glycine max Merr.	18/0		22/0	
Lathyrus odoratus L.				
var. Bridal Veil	8/8	M	6/6	m, LN
var. Cardinal	7/7	M	7/7	m, LN
var. Treasure Island	8/8	M	9/4	m, LN
Lens esculenta Moench.	16/16	VC, s	17/17	m, S
Lupinus albus L.	10/10	M, NS	10/1	VC
L. hirsutus L.	10/10	M, NS	10/0	
Medicago lupulina L.	10/10	M	10/10	m
M. sativa L.	10/6	M	10/0	
Melilotus alba Desr.	18/18	M	18/8	m
Phaseolus aureus Roxb.	15/1	VC	14/13	RS, NS
P. vulgaris L.				
var. Early Golden Cluster	15/15	M	15/6	m
var. Great Northern U. of Idaho No. 1	12/10	m	11/1	m
var. Ideal Market	10/0		11/0	
var. Kentucky Wonder	8/8	m	7/0	
var. Navy Robust	7/7	YL, m	9/0	
var. Red Kidney	8/8	YL, m	9/6	m
var. Red Valentine	16/16	M	11/11	m
var. Robust	9/9	YL, m	9/9	m
var. Stringless Refugee	9/9	M	9/5	m
var. Stringless Refugee Green Pod	9/9	M	9/4	m
var. U. S. No. 5 Refugee	12/9	YL, m	12/8	m
var. Unrivalled Wax	12/12	YL, m	12/6	m
Pisum sativum L.				
var. Alaska	15/10	M	17/15	m, LN
var. Dwarf Alderman	30/28	M	30/30	LN
var. Dwarf Telephone	28/26	M	30/30	LN
var. Hundredfold	39/38	M	36/35	LN
var. Laxton Progress	26/20	M	23/23	LN
var. Little Marvel	16/16	M	18/18	LN
var. Nott's Excelsior	18/18	M	20/20	LN
var. Perfection	23/23	M	29/29	LN
var. Potlatch	21/21	M	18/18	LN
var. *arvense* Poir. Canada White	20/20	M	19/18	m, LN

TABLE 1.—(*Continued*)

Host plant	Pea-mottle virus		Pea-wilt virus	
	Plants tested[a]	Symptoms[b]	Plants tested	Symptoms
Trifolium hybridum L.	5/5	M	5/5	None
T. incarnatum L.	10/10	m, VC	10/10	VC
T. pratense L.	10/10	M	10/10	m, VC
T. repens L.	10/10	M	10/10	M
Vicia faba L.	25/18	M, ns	23/13	m, RS
V. sativa L.	16/16	m, NS	16/16	m, VC
Vigna sinensis (L.) Endl.	30/0		50/38	m, BLL
Liliaceae				
Lilium formosanum Stapf.	10/0		10/0	
Plantaginaceae				
Plantago lanceolata L.	1/0		1/0	
P. major L.	1/0		1/0	
Polygonaceae				
Rumex acetosella L.	1/0		1/0	
Scrophulariaceae				
Antirrhinum majus L.				
var. Giant Crimson	8/5	M	8/0	
var. Giant White	8/3	M	8/0	
Solanaceae				
Datura stramonium L.	5/0		5/0	
Lycopersicon esculentum Mill.	15/0		15/0	
Nicotiana glutinosa L.	35/0		40/0	
N. tabacum L.	35/0		45/0	
N. rustica L.	5/0		5/0	
N. sylvestris Spegaz. and Comes	20/0		20/0	
Solanum nigrum L.	5/0		5/0	

[a] The numerator indicates the number of plants inoculated, denominator indicates the number of plants diseased.

[b] M = mottling; S = streak; YL = systemic yellow lesions; NS = necrotic spotting; VC = vein clearing; RS = ring spotting; LN = basal leaf wilting and necrosis; BLL = brown local lesions. Similar descriptions with small letters indicate these symptoms were mild.

mottle virus, caused typical symptoms of streak in Dwarf Telephone and Hundredfold peas (Fig. 1, *C*, e).

SUSCEPT RANGE

In order to obtain a better understanding of the relationship between the two viruses, a knowledge of their suscept ranges was needed. An attempt was made to test as far as possible those species and varieties of plants used by other investigators in their work with legume viruses. All plants were grown from seed in a greenhouse held at about 25° C. and fumigated regularly to destroy insects. Plants tested for susceptibility were young and in a stage of rapid growth. The test plants were dusted with carborundum powder and inoculated by the rubbing method. Inoculum was prepared from diseased tissue of young, rapidly growing plants by macerating it in a sterile mortar to which a few drops of tap water were added. A sterile cotton swab on a small stick was dipped in the inoculum and gently rubbed over the plant tissue, which was supported with a sterile pot label. This method of inoculation has been described in detail by Jones (8).

When plants with large leaves were inoculated, sterile gauze pads were used instead of the cotton swabs, and the leaves were supported in the hand. Immediately after inoculation the plants were rinsed with water from a sprinkling can in order to remove any toxic materials. From 2 to 5 leaves were inoculated, depending upon the size and growth habit of the plants. After a suitable incubation period, sub-inoculations were made to peas with juice from the tested host plants.

As is shown in table 1, pea-mottle virus infected plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae, while pea-wilt virus infected plants of the Leguminosae only.

SYMPTOMATOLOGY

Pea-mottle Virus. When pea plants were inoculated with pea-mottle virus, the developing leaves failed to open as readily as in healthy plants; and from 8 to 12 days after inoculation a fine clearing of veins appeared in the young foliage. The large veins were bleached and the network of fine veins stood out in contrast to the adjacent tissues (Fig. 1, *B*, b). Numerous, small, irregular, light-yellow spots were scattered over the youngest foliage. Infected plants were slightly stunted and lighter in color than healthy plants. The first two leaves that developed after the mottling was noticeable were more severely affected than the succeeding ones in which the yellow spots coalesced to form large light-green areas (Fig. 1, *B*, c). The stipules showed the same type of mottle that was found in the leaves. Some varieties of plants outgrew the mottling before blooming. No symptoms were noticeable on the stems; likewise, no apparent effects were observed in the pods or seeds of infected plants.

On bean (*Phaseolus vulgaris* L.), pea-mottle virus produced light-yellow spots and clearing of veins (Fig. 2, *A*, a). These symptoms were uniform on the varieties that were tested. On Alsike clover (*Trifolium hybridum* L.), red clover (*T. pratense* L.) and white clover, light-yellow areas appeared between the veins. The symptoms in alfalfa were distinct, consisting of irregular streaks of yellowing along the veins and adjacent tissues. In some cases dark-green irregular patches of tissue became outlined with light-yellow margins. Infected spinach plants (*Spinacia oleracea* L.) became dwarfed and severely mottled, and infected cucumber (*Cucumis sativus* L.) showed light-yellow secondary lesions (Fig. 2, *B*, b, c).

Pea-wilt Virus. Symptoms produced by this virus in pea were noticeable in from 5 to 8 days following inoculation. The inoculated leaves wilted and died and the petioles shriveled, leaving the dead, dried leaves attached to the stem (Fig. 1, *C*, a). One or more of the adjacent, lower leaves also wilted and died. In most cases the tops of the plants appeared healthy, but in two varieties, Alaska and Canada White, a faint mottling developed and soon disappeared. The stems showed a faint grayish discoloration. Infected plants grew slowly and were dwarfed in comparison with healthy plants.

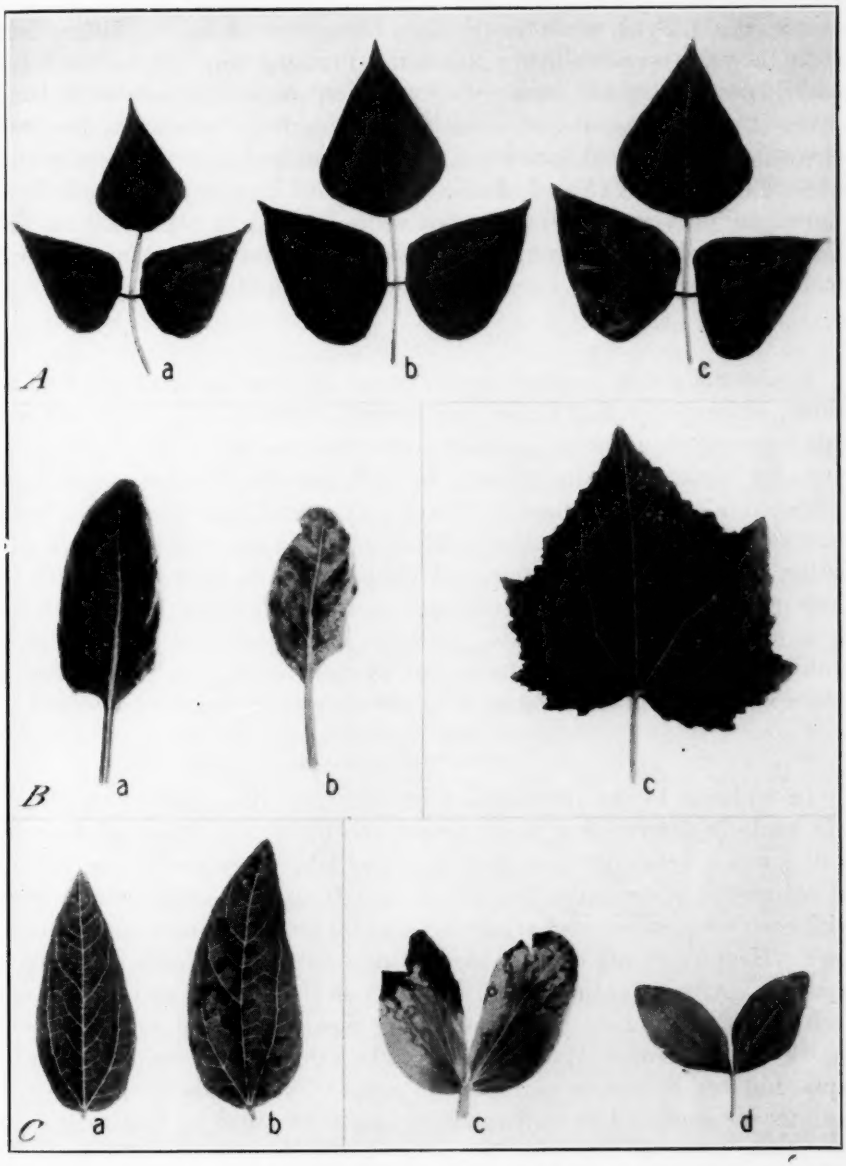

FIG. 2. Symptoms produced by separate virus constituents in several plants. *A*, a. Symptoms in Red Valentine bean caused by pea-mottle virus. b. Healthy Red Valentine bean. c. Diffuse mottle in Red Valentine bean caused by pea-wilt virus. *B*, a. Healthy spinach leaf. b. Mottling in spinach caused by pea-mottle virus. c. Symptoms in cucumber caused by pea-mottle virus. *C*, a. Healthy *Phaseolus aureus* leaf. b. Zonate necrotic spots in inoculated leaf of *P. aureus* caused by pea-wilt virus. c. Ring spotting and necrosis in broad bean caused by pea-wilt virus. d. Mosaic in broad bean caused by pea-mottle virus. (Photographs by Julian A. Carlile.)

In cowpea plants the inoculated primary leaves developed brown, local lesions (Fig. 1, *B*, a), while the trifoliate leaves showed isolated diffuse areas where the veins became slightly bleached. In mung bean (*Phaseolus aureus* Roxb.), necrotic zonate lesions were produced on the inoculated primary leaves (Fig. 2, *C*, b), and in some instances dark necrotic spots developed in systemically invaded leaves. A blotchy mosaic disease was produced in infected bean plants (Fig. 2, *A*, c). Most of the clovers that were infected showed only a very faint mottle, but white clover was noticeably mottled. Alsike clover showed no symptoms of disease, although the plants were systemically invaded by the virus.

HISTOPATHOLOGY

Epidermal strippings and cross sections of stems of Alsike clover, red clover, white clover, hop clover, and several varieties of pea infected with either pea-mottle virus or pea-wilt virus were examined under the microscope for intracellular inclusions. In each case the diseased material was compared with healthy tissue. Water and several other mounting media were used, but iodine-potassium iodide solution, in the proportion of 1 part iodine, 3 parts potassium iodide, and 150 parts water, gave the best results, staining the nuclei brown. Examinations failed to reveal inclusion bodies or unusual crystalline deposits. Calcium oxylate crystals were found in both healthy and diseased plants, but plate crystals, like those found in tobacco and other plants infected with certain viruses, were not observed.

TRANSMISSION STUDIES

In addition to the transmission experiments with dodder, an attempt was made to determine whether or not the pea aphid (*Macrosiphum pisi* Kalt.) was a vector of these viruses under laboratory conditions. Plants of red clover, white clover, hop clover, and Dwarf Alderman peas infected with each virus were caged separately and 30 healthy aphids added to each cage. Healthy plants of each species were treated similarly and used as controls. After a feeding period of 4 days on the diseased and healthy control plants, the aphids were transferred separately from each cage to 6 healthy, caged Dwarf Alderman peas. An attempt was made to place an equal number of insects on each pea plant. The aphids were allowed to feed for a period of 4 days after which they were killed by fumigation, and the plants removed to a greenhouse bench for observation. No symptoms of disease appeared on any of the test plants; likewise, all control plants remained healthy. Since pea-wilt virus did not produce mottling in pea, it seemed possible that the pea plants on which the aphids fed might have been infected without showing definite symptoms. To determine whether this was actually the case, a small portion of leaf tissue was removed from each plant, macerated in a mortar to which pea-mottle virus was added, and used as inoculum for healthy peas. Typical symptoms of pea-mottle developed in the inoculated plants instead of those of streak which would have been

the result had the plants been infected with both viruses. This result demonstrated that pea-wilt virus had not been transmitted to peas by the pea aphid.

A second test was made in the following manner: White clover plants infected with each virus were caged separately. One hundred healthy aphids were placed in each cage and allowed to feed 2 days. The aphids from each cage were then transferred separately to 25 individually caged, healthy Dwarf Telephone peas, 4 insects being placed on each plant. All insects were killed after a feeding period of 11 days and the plants removed to a greenhouse for observation. No symptoms of disease developed. As a further check on these plants, a portion of leaf tissue was removed from each plant of each lot, macerated together, and tested for virus by mechanical inoculation of healthy peas. These tests also were negative. The possibility existed that pea-wilt virus was present without showing symptoms of disease; in this event no streak would have appeared if pea-mottle virus had been absent. To test this possibility, leaf tissues from each pea plant in the pea-wilt-virus series were macerated together and pea-mottle virus was added to the plant juice. This mixture was used to inoculate healthy peas. Symptoms of pea-mottle developed in the inoculated plants instead of streak, which would have resulted if both viruses had been present in the inoculum. It is concluded from these results that the pea aphid was not a vector of these viruses.

PROPERTIES OF THE VIRUSES

Thermal Inactivation. Plants infected with each virus were ground separately in a sterile meat chopper, and the juice was extracted by passage through two layers of cheesecloth. The juice was placed in tightly stoppered test tubes (7×70 mm.) and completely immersed for 10 minutes in an electrically heated water bath. The water was stirred constantly by means of a stirring rod attached to an electric motor and the temperature was automatically controlled within $\pm 0.2°$ C. of the desired temperature. After a 10-minute immersion the test tubes were immediately plunged into ice water, and the cooled cell extract was used to inoculate healthy plants.

Infected plants of Dwarf Alderman, Dwarf Telephone, and Potlatch pea served as source plants for the viruses, and healthy plants of the same varieties were used as test plants. Between 15 and 20 plants were inoculated with each sample and each test was conducted on 3 different occasions. Attempts were also made to use cowpea and mung beans as test plants for measuring activity of the pea-wilt virus, but with erratic results. Therefore, in order to test for pea-wilt virus in heated samples, a small amount of pea-mottle virus was added to each sample after it had cooled. The test plants reacted by production of mottling symptoms when only the pea-mottle virus was present, but by production of streak when pea-wilt virus was also present. It was found that pea-mottle virus was inactivated by

exposure for 10 minutes to a temperature of 60–62° C., whereas pea-wilt virus was inactivated by exposure to a temperature of 58–60° C.

Tolerance to Dilution. Infectious plant juice was extracted, as previously reported, and diluted with distilled water in varying proportions up to 1 part in a million. Fifteen to 20 Dwarf Telephone peas were inoculated with each diluted sample. Pea-mottle virus was still infectious at a dilution of 1:10,000, but not 1:100,000, while pea-wilt virus was recovered at a dilution of 1:100,000 but not 1:1,000,000.

Resistance to Aging. Expressed plant juice from plants infected with each virus was stored separately in stoppered bottles at room temperature (about 25° C.) and used as inoculum for 18 to 20 Dwarf Telephone peas. Inoculations were made at 3-day intervals for 3 successive times; subsequent inoculations were made at irregular intervals up to 31 days after extraction. Both viruses withstood aging *in vitro* for 31 days. Neither virus was tested for resistance to aging for longer than this period.

Pea plants infected with pea-mottle virus, pea-wilt virus, and broad bean leaves infected with Zaumeyer and Wade's pea virus 2 were dried at room temperature, held in separate stoppered bottles, and tested for their resistance to aging in dried host tissue. The first inoculation with the dried material was made 7 days after the plants were cut, at which stage the plant tissue was thoroughly dry. A small amount of the dried material was soaked with a few drops of tap water in a sterile mortar, macerated and the plant extract used as inoculum for either Dwarf Alderman or Dwarf Telephone peas. Inoculations were made at 3-day intervals for 3 successive times, after which they were made at irregular intervals. It was found that all the tested viruses withstood aging in dried plant tissue for at least 31 days.

Filterability. Pea plants infected with each virus were separately ground in a meat chopper and the juice was passed through 2 layers of cheesecloth, after which it was centrifuged for 15 minutes at 3300 R.P.M. The liquid was decanted and filtered through a layer of medium-size Celite before passage through a Berkefeld W filter. A 1 cc. sample of the filtered pea juice from each series of plants was added to laboratory broth and thus shown to be free from contamination; subsequently, portions of the filtered plant juice were used as inoculum for 20 to 25 Dwarf Telephone pea plants. In one test, infection was obtained in all plants inoculated with filtered pea-

TABLE 2.—*Comparison of physical properties of pea-mottle virus and pea-wilt virus*

Virus	Thermal inactivation	Tolerance to dilution	Resistance to aging		Filterability
			in vitro	in dry host tissue	
Pea-mottle virus	60–62° C.	1:10,000	At least 31 days	At least 31 days	+
Pea-wilt virus	58–60° C.	1:100,000	At least 31 days	At least 31 days	+

wilt juice, but no plants became diseased when inoculated with pea-mottle filtrate. In a subsequent experiment it was shown that pea-mottle virus passed a Berkefeld W filter and infected inoculated plants. These results prove that both pea-wilt virus and pea-mottle virus pass Berkefeld W filters.

The physical properties of the two viruses are summarized in table 2.

DISCUSSION

The fact that Trifolium virus 1 has proved to be a mixture of two viruses was not altogether unexpected, having been suggested by Zaumeyer and Wade (25), who found that the so-called Trifolium virus 1 produced two types of symptoms on many bean varieties. In addition, they reported two different temperatures of virus inactivation and suggested that two viruses might be involved. They, however, did not separate the suspected constituents from the mixture. Pierce (13) found a mosaic infected red-clover plant in the field, the juice from which produced streak when transferred to pea. Inoculations of small-seeded broad bean with juice from the infected red clover resulted in local, necrotic lesions on inoculated leaves followed by a systemic mottling. When extract of the mottled tissue of broad bean was used to inoculate peas, only a mild mottling developed. Pierce considered the mottling virus to be identical with the one he previously had described and named bean virus 2, while the virus that produced local lesions in broad beans was named broad-bean local-lesion virus. Little is known about the latter virus and its reactions in pea. Pierce, however, suggested that when the mottling virus and broad-bean local-lesion virus were combined and used to inoculate pea, streak would be induced, but he cited no definite experimental evidence for this conclusion. It is believed that Pierce and the writer probably worked with the same virus complex, since the writer's original material was collected near Pullman, Washington, a few miles from the locality where Pierce found his infected red clover. This belief is supported by the fact that pea-mottle virus produces a faint mottle in broad beans, while pea-wilt virus causes the production of necrotic ringspots in the same plants (Fig. 2, *C*, c, d). However, bean virus 2 of Pierce was reported to be non-infectious for white clover and alfalfa and also differed from pea-mottle virus in its resistance to aging *in vitro* and in its tolerance to dilution, thus suggesting that the two viruses are different. The writer believes that Pierce's evidence for considering that bean virus 2 was a component of his red clover virus complex was inconclusive, since it was based only on his observations of the symptoms produced in Stringless Refugee Green beans by his broad-bean mosaic virus. It is the writer's belief that Pierce had the virus complex in red clover that has been classified by Weiss (18) as Trifolium virus 1, but that is shown in this paper to consist of two separate viruses. Pea-mottle virus is similar to the virus that Pierce found to become systemic in broad bean and that produced a mottling in peas, whereas the pea-wilt virus of the writer is probably closely related to Pierce's (13) broad-bean local-lesion virus.

Pea virus 2 of Zaumeyer and Wade (25) resembles pea-mottle virus in the symptoms it produces on many pea and bean varieties. A further similarity between these two viruses is their resistance to drying in host tissue. Other similarities between the two viruses can be found in their suscept range, although they differ somewhat in this respect. One point of difference between pea virus 2 and pea-mottle virus is the fact that pea virus 2 does not produce streak in peas when transferred to these plants in combination with the pea-wilt virus discussed in this paper.

Severe pea-mosaic virus previously described (6) resembles pea-mottle virus on the basis of suscept range, longevity *in vitro* and dried tissue, tolerance to dilution, and heat inactivation. This suggests that these two viruses may be related.

Since it was shown that pea-mottle virus was infectious for plants in 5 different families, the question arises whether this virus is not related to one of the strains of cucumber-mosaic virus that have been found infecting peas (20, 23). Whipple and Walker (20) have shown that 2 cucumber viruses are infectious for a large number of plants in several plant families, including peas and other legumes. Pea-mottle virus has failed to infect corn (*Zea mays* L.), zinnia (*Zinnia elegans* Jacq.), or solanaceous plants, which are generally considered susceptible to infection with cucumber-mosaic virus. Likewise, the symptoms produced by the two viruses studied by Whipple and Walker in peas, beans, and cucumbers differ from those produced by pea-mottle virus.

Not only was it true that pea-mottle virus was infectious for plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae, whereas pea-wilt virus was infectious only for plants in the Leguminosae, but also, in all cases where plants were susceptible to both viruses, a more severe disease was produced by pea-mottle virus than by pea-wilt virus, except in the case of white clover, where similar symptoms were produced by both.

The evidence at hand points to the conclusion that the two viruses discussed in this paper are distinct from each other and that they show characteristics that distinguish them from any of the viruses previously given scientific names according to the system of nomenclature outlined by Holmes (5). In accordance with this system, pea-mottle virus may be referred to as *Marmor efficiens* n. sp., from Latin *efficiens* meaning effective, in reference to the ability of this virus to cause mottling in peas in contrast with the inability of pea-wilt virus to produce such chlorotic symptoms in tested varieties of this host other than Alaska and Canada White. Probable synonyms are: broad-bean-mosaic virus of Pierce (13), which was present in his red-clover-mosaic complex; severe pea-mosaic virus, previously described by Johnson and Jones (6); possibly also pea virus 2 of Zaumeyer and Wade (25). Pea-wilt virus may be referred to as *M. repens* n. sp. The specific name is taken from Latin *rĕpens* (not from *rēpens*) and means unlooked for, in reference to the unexpected discovery of a second virus in

the original complex. Broad-bean local-lesion virus of Pierce (13) may be considered as a probable synonym.

SUMMARY

It has been shown that white-clover mosaic, a disease previously regarded as caused by a single virus classified as Trifolium virus 1, was actually induced by a mixture of two distinct viruses: namely, pea-mottle virus and pea-wilt virus. Their separation was accomplished because pea-mottle virus alone was transmitted by dodder (*Cuscuta campestris*), while pea-wilt virus infected cowpea (*Vigna sinensis*), a plant resistant to pea-mottle virus.

When pea-mottle virus in combination with pea-wilt virus was transferred to peas, streak was produced and resulted in death of the plants in a manner similar to that produced by the Trifolium virus 1 complex.

Pea-mottle virus alone produced a systemic mosaic disease when transferred to several pea varieties, and was infectious for plants in the Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Scrophulariaceae, and Leguminosae. In the tests conducted, pea-wilt virus was infectious for plants in the Leguminosae only, and produced no mottling in pea varieties, except Alaska and Canada White, where a very mild mosaic was produced. In general, plants infected with pea-mottle virus were more severely affected than when similar plants became infected with pea-wilt virus. No intracellular inclusion bodies could be detected in plants infected with either virus, and no virus transmission was obtained by allowing the pea aphid (*Macrosiphum pisi*) to feed on infected and healthy pea plants in succession.

Pea-mottle virus was inactivated by exposure for 10 minutes to a temperature of 60–62° C., whereas pea-wilt virus became inactive between 58–60° C. Pea-mottle virus was recovered in a dilution of 1:10,000 in water, and pea-wilt virus in a dilution of 1:100,000. Both viruses withstood aging *in vitro* and in dried host tissues for at least 31 days and were filterable through a Berkefeld W filter.

The name **Marmor efficiens** n. sp. is suggested for pea-mottle virus and **M. repens** n. sp. for pea-wilt virus.

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NEW PHYSIOLOGIC RACES OF TILLETIA TRITICI AND T. LEVIS[1]

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INTRODUCTION

In a previous paper the writers (9) discussed the status of physiologic-race identification in *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn and emphasized the need for a standard system on which to base the identification of races, particularly as related to the breeding and distribution of resistant varieties of wheat in the United States. At that time 11 races of *T. tritici* and 8 of *T. levis* were classified on the basis of the resistant (R), intermediate (I), and susceptible (S) reaction of certain winter- and spring-wheat varieties. Since the publication of that paper, additional collections of *T. tritici* and *T. levis* from all of the principal wheat-growing States have been tested and 5 additional races of these fungi have been identified and numbered, consecutively, in accordance with the original system. Also, studies and observations have been made on race-differentiating characters other than pathogenicity. The results of these studies and the extended race classification are here presented.

MATERIALS AND METHODS

All of the previously classified physiologic races of *Tilletia tritici* and *T. levis* (9) and many collections of both species obtained from commercial wheat fields in the principal wheat-growing sections of the United States were used in these studies. The differential varieties were the same as those used in the former studies (9), except that Martin (C.I.[3] 4463) and White Odessa (C.I. 4655) were added to the winter-wheat group, and Mindum (C.I. 5296) was omitted from the spring-wheat group. Hybrid 128 was used throughout as a susceptible winter-wheat check.

The tests on winter wheats were made at Pullman, Washington, and those on spring wheats at Bozeman, Montana. In 1940 an additional nursery was grown at Aberdeen, Idaho. The previously described (9) technique for conducting the experiments and analyzing the data for race identification was employed in these studies. Race differentiation by means other than pathogenicity is mainly a matter of observation, and any special techniques used in this phase of the investigations are described along with the presentation of results.

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Stations of Idaho, Montana, Oregon, Utah and Washington.

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EXPERIMENTAL RESULTS

New Physiologic Races

Three new races of *Tilletia tritici* and 2 of *T. levis* have been identified on the basis of results obtained from pathogenicity tests made during the 4 years, 1937–1940. The percentages of infection obtained on the differential varieties with these races each year are summarized in table 1; and the reaction of the differential varieties (host testers) to these races and those previously classified is shown in table 2. Although the identity of the new races is based primarily on the reaction of the winter wheats, some of them differ also in pathogenicity on the spring wheats. As shown in tables 1 and 2, T–12 is differentiated by the susceptibility of Hohenheimer, Albit, and White Odessa, and the intermediate reaction of Martin. This is the only race that infects all of these varieties. Race T–13 is differentiated by the susceptibility of Ridit, Hussar, Albit, and White Odessa, and, likewise, this is the only race that infects all of these particular varieties (Tables 1 and 2). On the other hand, T–14 is differentiated by the susceptibility of Albit and White Odessa only (Table 2), whereas all the other races that infect these two varieties also infect one or more other varieties. The identity of L–9 is based on the susceptibility of Ridit and Albit, while L–10 is differentiated by the intermediate reaction of Ridit (Tables 1 and 2). In this analysis it is notable that four of the new races can be identified by the reaction of the host testers used in previous studies, while the identity of one of these races (T–14) necessitates the use of Martin and White Odessa also.

As shown in table 1, there was some degree of variability in the pathogenicity of the new races in the different seasons. Relatively low percentages of infection were obtained in 1938 with T–12, T–13, T–14, and L–9, due, apparently, to unfavorably high temperature during the infection period. Percentages of infection with these races in that year are, therefore, omitted from the averages. Apparently, these conditions did not affect the virulence of L–10, and the percentages of infection with this race are included in the averages.

These data support Aamodt's (1) suggestion that there are differences in the optimum temperature requirements for infection by different races. Generally, however, relatively low temperature favors infection by the bunt fungi; and, with the exception referred to, there is a notable consistency in the reaction to the races shown in table 1, particularly of the varieties that identify the various races. For example, the identity of T–12 is based on the S reaction of Hohenheimer and Albit, and the percentage of infection on both varieties is well within that infection class each year. In the case of T–13, which is identified by the S reaction of Ridit and Albit, the percentages of infection on Albit are well within that infection class, while on Ridit they are just slightly above the minimum for the susceptible class. Nevertheless, it seems noteworthy that for three consecutive years the S

TABLE 1.—*Percentage of bunt produced on differential varieties by three physiologic races of Tilletia tritici and two of T. levis*

Species	Race No.	Year	Smutted heads in										
			Hybrid 128 (C.I. 4512)	Ridit (C.I. 6703)	Oro (C.I. 8220)	Hohenheimer (C.I. 11458)	Hussar (C.I. 4843)	Albit (C.I. 8275)	Martin (C.I. 4463)	Wh. Odessa (C.I. 4655)	Ulka (C.I. 11478)	Marquis (C.I. 3641)	Canus (C.I. 11637)
			P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Tilletia tritici	T–12	1937	89.0	0.7	1.4	73.0	8.0	92.0	.....	.....	73.0	11.0	0.1
		1938[a]	85.0	1.0	2.0	10.0	3.0	59.0	.....	.....	86.0	9.0	0.0
		1939	.....	0.0	0.0	69.0	1.0	75.0	23.0	79.0	.....	.....	.....
		1940	95.0	2.0	1.0	58.0	1.0	76.0	31.0	89.0	96.0	18.0	0.0
		Av.	92.0	1.3	1.2	66.6	3.3	81.0	27.0	84.0	85.0	12.6	0.0
Do	T–13	1938	70.0	15.0	1.0	0.0	8.0	33.0	.....	.....	.....	15.0	23.0
		1939	92.0	43.0	4.0	0.0	48.0	85.0	.....	.....	.....	.....	.....
		1940	66.0	45.0	1.0	0.0	55.0	71.0	0.0	85.0	18.0	26.0	27.0
		Av.	79.0	44.0	2.5	0.0	51.5	78.0	0.0	85.0	.....	20.5	25.0
Do	T–14	1938	45.0	2.0	3.0	0.0	0.1	24.0	2.0	65.0	.....	.....	.....
		1939	95.0	0.3	1.0	0.0	3.0	76.0	3.0	86.0	.....	.....	.....
		1940	95.0	1.0	1.0	0.0	9.0	86.0	0.0	93.0	7.0	2.0	0.0
		Av.	95.0	0.6	1.0	0.0	6.0	81.0	1.5	89.5	7.0	2.0	0.0
Tilletia levis	L– 9	1937	67.0	52.0	9.0	1.0	20.0	45.0	.....	.....	.....	.....	.....
		1938	76.0	52.0	6.0	0.0	6.0	23.0	.....	.....	.....	.....	.....
		1939	88.0	38.0	4.0	0.0	31.0	66.0	.....	.....	.....	.....	.....
		1940	87.0	63.0	1.0	0.0	58.0	64.0	0.0	82.0	51.0	23.0	31.0
		Av.	80.6	50.6	4.6	0.3	36.3	58.3	0.0	82.0	51.0	23.0	31.0
Do	L–10	1938	92.0	46.0	9.0	0.0	0.0	0.0	.....	.....	90.0	16.0	18.0
		1939	64.0	22.0	13.0	0.0	0.0	0.0	.....	.....	.....	.....	.....
		1940	90.0	38.0	7.0	0.0	0.0	0.0	0.0	0.0	99.0	26.0	37.0
		Av.	82.0	34.3	9.6	0.0	0.0	0.0	0.0	0.0	94.5	21.0	27.5

[a] The data for 1938 are not included in the averages, except for L–10, which apparently was not affected by conditions that were unfavorable for infection by other races.

TABLE 2.—*Reaction of physiologic races of Tilletia tritici and T. levis to the differential varieties*

Race No.	Hybrid 128	Ridit	Oro	Hohenheimer	Hussar	Albit	Martin	White Odessa	Ulka	Marquis	Canus	Mindum
T– 1	S[a]	R	R	R	R	R	R	R	S	I	R	R
T– 2	S	R	R	R	R	R	R	R	S	R	R	S
T– 3	S	R	R	R	R	R	R	R	S	S	S	I
T– 4	S	R	R	R	R	I	S	S	S	S	R	I
T– 5	S	R	R	R	R	I	S	S	S	S	S	I
T– 6	S	R	R	R	R	S	S	S	S	S	R	I
T– 7	S	R	R	R	I	S	S	S	S	S	I	I
T– 8	S	R	R	R	S	S	S	S	S	S	S	I
T– 9	S	R	R	I	R	R	R	R	S	I	R	I
T–10	S	R	R	S	R	R	R	R	R	I	R	R
T–11	S	S	R	R	R	R	R	R	I	S	S	I
T–12	S	R	R	S	R	S	I	S	S	R	R	...
T–13	S	S	R	R	S	S	R	S	...	I	I	...
T–14	S	R	R	R	R	S	R	S	S	R	R	...
L– 1	S	R	R	R	R	R	R	R	S	I	R	I
L– 2	S	R	R	R	R	R	R	R	S	S	R	I
L– 3	S	R	R	R	R	R	R	R	S	S	S	I
L– 4	S	R	R	R	R	S	S	S	S	I	R	I
L– 5	S	R	R	R	R	S	S	S	S	S	S	I
L– 6	S	R	R	R	I	S	S	S	S	S	S	I
L– 7	S	R	R	R	S	S	S	S	S	I	S	I
L– 8	S	R	S	R	R	R	R	R	S	S	S	I
L– 9	S	S	R	R	I	S	R	S	...	...	...	...
L–10	S	I	R	R	R	R	R	R	...	...	...	...

[a] R = Resistant (0–10 per cent infection); I = Intermediate (11–40 per cent infection); S = Susceptible (41–100 per cent infection).

reaction on Ridit was maintained by a slight margin. The S reaction of Albit and White Odessa and the R reaction of Martin, Hussar, and Ridit differentiates T–13 from the other races; and the percentages of infection produced by this race, shown in table 1, are well within their respective classes.

Slight discrepancies are apparent in the results with L–9, which is identified by the S reaction of Ridit and Albit. The percentage of infection on Ridit was slightly below the minimum for the susceptible infection class in 1939 but well above the minimum in the other 3 years. Also, the reaction of Hussar, although not important in the identification of this race, averaged intermediate (I) over a 3-year period but was susceptible (S), in 1940. These results, and those obtained with other races, indicate that the intermediate (I) reaction class is highly variable and, consequently, may not be entirely reliable in race identification, particularly when the percentage of bunt usually obtained is near the minimum or the maximum for this class. Nevertheless, there is a definite intermediate reaction, as exemplified by T–9 on Hohenheimer, which it seems desirable to recognize, even though several years' results may be required to establish such a reaction.

The original source of the inoculum of each of the 5 new races of *Tilletia*

tritici and *T. levis* is shown in table 3. It will be noted that T–12 came from Oregon, T–13 and L–10 from Washington, and T–14 and L–9 from Idaho. The original source of T–13 is of particular interest and is described later in this paper in discussing the stability of physiologic races.

TABLE 3.—*Original source of the inoculum of the five new races of Tilletia tritici and T. levis*

Species	Collection No.	Race No.	Source
Tilletia tritici	268	T–12	From a commercial wheat field in Umatilla Co., Oreg. Collected in 1934 by J. F. Martin who made preliminary tests in 1935 and 1936 on the differential hosts at the Pendleton Field Station, Pendleton, Oreg.
	329	T–13	A selection of T–11 from the variety Albit in the physiologic race nursery at Pullman, Wash., 1937. Collected by C. S. Holton and H. A. Rodenhiser.
	334	T–14	From a commercial wheat field near Cottonwood, Idaho. Collected in 1936 by W. M. Bever who made preliminary tests on the differential hosts at the Idaho Agricultural Experiment Station, Moscow, Idaho.
Tilletia levis	331	L– 9	From a commercial field of Ridit near Lewiston, Idaho. Collected in 1936 by C. S. Holton.
	92	L–10	From an increase plot of Ridit on the Washington Agricultural Experiment Station, Pullman, Wash. Collected in 1932 by C. S. Holton.

Distinguishing Characters of Physiologic Races Other than Pathogenicity

It is recognized that physiologic races of *Tilletia tritici* and *T. levis* may exhibit differences in characteristics other than pathogenicity. These differences, which may be either morphological or physiological, include such characteristics as size and shape of bunt balls, size of chlamydospores, prominence of spore wall reticulations, spore size and color, relative capacity for stunting the host, and the tendency for partial smutting of the wheat spikes. Studies have been made of these characters of some or all of the races now recognized and the results are here presented.

One of the principal secondary criteria for distinguishing between races of the bunt fungi is characteristics of the bunt balls. The bunt ball characteristics of the 24 races of *Tilletia tritici* and *T. levis* on both spring and winter wheat varieties listed in table 2 were studied. Noteworthy differences in size and shape of the bunt balls were observed in certain races but none of these was essentially different from those reported by other workers (3, 4, 6, 12).

Bunt balls of different physiologic races may differ also in hardness and in rate of water absorption. Those of the so-called dwarf bunt are char-

acteristically small, hard, and almost spherical (15). Usually, individual bunt balls of this race are sufficiently hard to be difficult to crush with the fingers. The chlamydospores, however, appear very dry and powdery in contrast to the oily nature of those of other races. This difference probably accounts for the rapidity with which the balls of the dwarf bunt absorb water. Repeated tests have shown that the pericarp ruptures and the spores are exuded, almost invariably, within a few minutes after bunt balls of this race come in contact with water, while those of other races require from several hours to several days to rupture and exude spores, unless their pericarps are cracked. In tests with 100 bunt balls of each of 6 races of *Tilletia tritici*, approximately 75 per cent were intact after 18 hours. During this time the average weight of each bunt ball had more than doubled due to absorbed water. In similar tests with the same number of bunt balls of each of four races of *T. levis* approximately 90 per cent of those of each race were intact at the end of 18 hours, and the average weight of each was more than doubled by water absorption. In tests with many balls of the dwarf bunt, represented by specimens from different localities, the majority broke within 2 or 3 minutes and only one was observed that required longer than 10 minutes for the pericarp to break after coming in contact with water. In this case, 58 minutes elapsed before the spores began to exude. Obviously, therefore, the dwarf bunt race of *T. tritici* is distinctly different from other races of this species and of *T. levis* in respect to water-absorptive properties of the bunt ball. The exact nature of this difference has not been determined; possibly it is connected with a difference in the oil content of the spores.

Prominence of the reticulations of the chlamydospore walls and size of the chlamydospores are two of the more common morphological characters by which physiologic races of the bunt fungi may be distinguished. Such differences have been reported by several investigators (3, 6, 12, 15). The writers also have observed that some of the races studied differ in these characteristics. For example, chlamydospores of T–8 appear almost smooth, because of their extremely shallow reticulations, whereas those of the dwarf bunt seem spiny because of their prominent reticulations. Intergrading types, also, exist, typically represented by the reticulations of T–9 and T–10, thus completing a series from the T–8 type to that of the dwarf bunt. Apparent differences between reticulations of the spores of other races have been observed, but, because of the intergrading types, finer distinctions than those described above usually cannot be made with certainty. Similar differences were observed by Gassner (5) who established *Tilletia tritici* var. *intermedia* on the basis of the intermediate character of the reticulations of the chlamydospores. Spore size is considered to be of limited value in distinguishing between races of the bunt fungi. Usually measurements of many spores are necessary so that statistical analysis may be applied in order to show significant differences. Differences in spore size among different races have been demonstrated by several workers (6, 11, 13). The

writers measured the diameter of 100 chlamydospores of each of 12 races of *Tilletia tritici* and 8 of *T. levis*. In the former species the diameter of the spores ranged from averages of 17.1 μ in T–3 to 19.7 μ in T–10. In *T. levis* the average spore diameters ranged from 16.3 μ in L–1 to 17.5 μ in L–7. These data indicate that the spores of the *T. levis* races, on the average, are smaller than those of the *T. tritici* races. Obviously, therefore, some races of the bunt fungi differ in size of chlamydospores; for the most part, however, these small differences cannot be used to any particular advantage in the differentiation of races.

Chlamydospore color, also, is recognized as a possible means of distinguishing between races of *Tilletia tritici* and *T. levis*. On this basis Spangenberg and Gutner (13) described 4 races of *T. tritici* and 3 of *T. levis*. In the former species the chlamydospores were classified as dark-brown, light-brown, typical, and whitish-brown, while in the latter they were classified as dark-brown, light-brown, and greyish-brown. In studies made by the writers on 25 races of both species, it was found that, in general, the spores of *T. levis* were of lighter color than those of *T. tritici*. One race of *T. levis* (L–4, represented by Collection 250 from Griffin, Ind.) was observed to have spores conspicuously lighter in color than all of the other races. But with this exception, race differentiation on the basis of spore color was unsuccessful.

Physiologic races may differ also in their ability to stunt the host, as shown by other investigations (6, 8, 15) and by the writers. Previously (6), it was reported that T–9 and T–10 differed in their capacity for stunting Hybrid 128 and Hohenheimer. Subsequent observations have confirmed this. Also, collection No. 258 of *Tilletia tritici*, which has about the same pathogenicity as T–1, is readily distinguished from T–1 and other races by its ability to stunt Hybrid 128 and Ulka. Dwarf bunt causes the greatest stunting of any known race. Differences, also, were observed in the stunting caused by *T. levis*, notably L–8. Race identification by this means, however, is limited to the extreme types cited above.

Studies also were made to determine whether reduction in height results from reduction in length of the internodes, as reported by Barrus (2) and Mourashkinsky (7), or in number of internodes, as found by Viennot-Bourgin (14). The results of these studies are presented in table 4. It will be noted that there was no significant difference in the number of internodes on infected and bunt-free plants of the same variety. Marked differences were exhibited, however, in the internode length of infected and bunt-free plants. In Hybrid 128, the internodes of the bunt-free plants averaged 17.2 cm. in length, while those of plants infected by T–13 and collection No. 258 averaged 12.8 and 6.9 cm., respectively. With Albit and Triplet, comparable results were obtained. Thus, in these varieties reduction in height of plants infected by these races is attributable to shortening of the internodal length, which agrees with the results reported by Barrus (2) and Mourashkinsky (7).

Incomplete smutting of infected heads also may be a distinguishing characteristic of physiologic races. Collection 13 of *Tilletia tritici* and T–8 both exhibit this phenomenon on Hohenheimer and Hosar, respectively. A summary of the data obtained from a study of heads partly smutted by these two races is presented in table 5. On Hohenheimer, infected by collection No. 13, 90.4 per cent of the infected heads were partly smutted and only 9.6 per cent totally so. Usually there were only one or a few bunt balls per head. Different degrees of incomplete smutting of Hohenheimer by this race are shown in figure 1. For the most part, however, there were

FIG. 1. Partially-smutted heads of Hohenheimer wheat caused by Collection 13 of *Tilletia tritici*. (1) One bunt ball; (2) Two bunt balls; (3) Five bunt balls; (4) Completely smutted head.

fewer bunt balls than sound kernels, the respective percentages being 60.2 and 39.8 (Table 5). As shown further in table 5, although 21.9 per cent of the heads were infected, only 8.7 per cent of the kernels were destroyed. Similar results were obtained with T–8 on Hosar (Table 5). Both of these races produced only totally-smutted heads on Hybrid 128.

The pathogenicity of collection No. 13 of *Tilletia tritici* on the differential varieties places it in the category of T–9, but its incomplete smutting proclivity on Hohenheimer readily distinguishes it from that race, which produces totally smutted heads on this variety.

Two other criteria for the separation of races of the bunt fungi are recognized. One of these is the capacity to intensify the purple pigmentation in the glumes of Ulka. This is especially true of T–8, T–10, and T–11,

TABLE 4.—*Average number and length of internodes in bunt-free and infected culms of three varieties of wheat*

Variety	Race	Number of culms	Internodes per culm	Length of internodes
			No.	*cm.*
Hybrid 128	T–13	100	5.8	12.8
	Collection 258[a]	100	5.5	6.9
	Bunt-free	100	5.8	17.2
Albit	T–13	100	5.8	13.7
	Bunt-free	100	5.7	16.9
Triplet	Dwarf bunt	124	4.6	7.6
	Bunt-free	95	5.0	18.3

[a] Collection No. 258 of *Tilletia tritici*. No race number has been assigned.

TABLE 5.—*Summary of data on incomplete smutting caused by two physiologic races of Tilletia tritici on two wheat varieties*

Variety	Race	Smutted heads	Heads smutted		Kernels		Smutted	
			Partly	Totally	Sound	Smutted	Heads	Kernels
		Number	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Hohenheimer (C.I. 11458)	Coll. 13[a]	104	90.4	9.6	60.2	39.8	21.9	8.7
Hosar (C.I. 10067)	T–8	113	95.0	5.0	62.8	37.2	43.1	16.0

[a] Collection 13 of *Tilletia tritici*. No race number has been assigned.

and to a lesser extent T–3 and T–5. Only race 9 of *Tilletia levis* expressed this characteristic. The other criterion is the capacity to cause excessive tillering of infected plants. This is particularly characteristic of the dwarf bunt race (12). Although definite counts have not been made of the number of tillers produced on plants infected by this and other races, enough observations have been made under different environmental conditions and on different varieties to establish the fact that this race is clearly different in its capacity to stimulate excessive tillering.

STABILITY OF PHYSIOLOGIC RACES

In the bunt fungi the term physiologic race is used to designate chlamydospore populations that differ from each other clearly, and fairly consistently, in one or more ways, but mainly in pathogenicity. Variability in the pathogenicity of a race may result either from environmental influence on the host or pathogen (1, 10) or from changes in the genetic constitution of the chlamydospores. Since the chlamydospores are diploid, considerable variation may possibly occur in one or more of the differentiating characters in succeeding generations, unless the spores are homozygous, in which case variability might arise as a result of mutation. This is an important consideration in race classification, as the identification of

TABLE 6.—*Summary of data indicating heterozygosity of the chlamydospores of race T–11, on the basis of the reaction of differential varieties in 1935–1940*

Collection No.	Race	Year	Inoculum from	Smut percentages in							
				Hybrid 128	Ridit	Oro	Hohenheimer	Hussar	Albit	Martin	White Odessa
	T–11	1935	Ridit	90.0	55.0	1.0	0.0	0.0	2.0	.....	.....
	"	1936	"	77.8	56.4	2.8	0.0	0.0	11.2	.....	.....
	"	1937	"	75.3	44.1	4.2	0.0	16.7	27.4	.....	.....
	"	1938	"	59.0	50.0	0.0	0.0	0.0	9.0	.....	.....
329	"	1938	Albit	70.0	15.0	1.0	0.0	8.0	33.0	.....	.....
330	"	1938	Hussar	49.0	9.0	0.0	0.0	7.0	19.0	.....	.....
	"	1939	Ridit	.....	52.5	2.0	0.0	4.8	15.1	.....	.....
329	T–13	1939	Albit	92.0	43.0	4.0	0.0	48.0	85.0	.....	.....
330	"	1939	Hussar	.....	58.0	6.3	0.0	54.0	81.5	.....	.....
	T–11	1940	Ridit	85.0	47.0	2.0	3.0	0.0	1.0	1.0	5.0
329	T–13	1940	Albit	66.0	45.0	1.0	0.0	55.0	71.0	0.0	85.0

new races is contingent upon a relatively high degree of constancy in the characteristics of known races. The writers have attempted to determine, insofar as is possible, the stability under field conditions of the physiologic races of *Tilletia tritici* and *T. levis* classified in table 2.

Lack of purity in chlamydospore populations of *Tilletia tritici* and *T. levis* may be due either to mechanical mixture, in which case genotypically different chlamydospores would be included in one collection, or to heterozygosity of the spores, resulting from hybridization. Obviously, mechanical mixtures make hybridization possible, and a heterozygous condition may result. In race identification, either condition is a complicating factor that might be obscured for a number of years. Fortunately, this factor apparently is not often encountered, as is indicated by the fact that almost all of the races identified by the writers have remained remarkably constant in pathogenicity on the winter-wheat differential varieties for several years in tests at Pullman. However, at least one race, T–11, was not constant (Table 6). This race is characterized by the susceptibility (S) of Ridit and the resistance (R) of the other winter-wheat differential varieties (Table 2). The typical reaction was obtained in 1935, but, in 1936, the percentage of infection on Albit was slightly above the upper limits of the resistant (R) class; and, in 1937, there was a further increase in virulence on Albit and also on Hussar. Studies were undertaken to determine whether the apparent change in pathogenicity of T–11 was attributable to mechanical mixture with another race, to a change in the genetic constitution, or to the influence of seasonal differences in the environment.

In 1937, inoculum was taken from Ridit, Albit (Collection 329), and Hussar (Collection 330) and used to inoculate the winter-wheat differential varieties. Although the infection percentages for 1938 were relatively low, because of unfavorable conditions for infection, they indicated that 2 distinct types of pathogenicity were represented in the 3 collections. Again, inoculum was taken from the 3 varieties, as indicated above and shown in table 6, and tests were made in 1939. The results show clearly that T–13, a new race, characterized by the susceptible (S) reaction of Ridit, Hussar, and Albit, was separated from the original T–11. The reaction of the differential varieties to T–13 was the same in 1940 as in 1939. Its contrast to the T–11 reaction is shown by the results obtained with inoculum of T–11 from another source.

The exact origin of T–13 cannot readily be explained by the data at hand. It appears, however, that the change was of a genetic nature, due either to segregation of factors for pathogenicity in heterozygous spores of the original T–11 or to mutation.

DISCUSSION

The importance of physiologic specialization of *Tilletia tritici* and *T. levis* as a factor in the problem of control of bunt of wheat through resistant varieties is too well understood to require elaboration here. Obviously,

however, the identification of new races has a direct relation to the complexity of this problem. For example, prior to the identification of T–12, no race capable of infecting both Albit and Hohenheimer was known, although each of these varieties was susceptible to other races. Therefore, a cross between these two varieties might have been expected to produce segregates that would be resistant to all known races. The discovery of T–12, however, eliminated that expectation because of the common susceptibility of Albit and Hohenheimer to this race. Similarly, Albit and Ridit are now known to have common susceptibility to races T–13 and L–9, whereas, formerly, this situation was not recognized. In other words, an increase in the number of known races serves to reduce the number of combinations of apparently highly bunt-resistant varieties. Consequently, it seems desirable to identify and recognize the greatest possible number of existing races of *T. tritici* and *T. levis* and thereby enhance the production of varieties with the highest possible resistance.

The differentiation of physiologic races of the bunt fungi by criteria other than pathogenicity seems to have limited application, insofar as the majority of races used in these studies are concerned. Certain notable examples were observed in which races were readily distinguished by bunt-ball characters, chlamydospore markings, effect on host development, and other means, but these differences apparently have no direct relation to the problem of bunt control. In the final analysis, at least from the practical point of view, pathogenicity is the most important consideration; consequently, this criterion remains the primary basis for race identification.

The reliability of a standard system for the identification of physiologic races of *Tilletia tritici* and *T. levis* depends largely on the stability of races after they have been identified and numbered. In other words, if the pathogenicity of a race is to be of any value for comparison with new, unidentified races, it should remain fairly constant in its reaction to a given set of differential varieties. The possibilities for hybridization and mutation in the bunt fungi are well known; because of these possibilities considerable variability in pathogenicity and other characters of a given race might be expected, unless the original inoculum consisted of homozygous spores. In dealing with the large number of races and collections used in these studies, efforts were made to obtain reasonably constant pathogenic reactions over a period of three years before designating a collection as a race. That a high degree of success was obtained seems to be indicated by the fact that of 19 races identified in 1937 only one (T–11) failed to maintain its identity. In this case a new race (T–13) was derived from a known race and there is evidence that the original race was retained. As already pointed out, the origin of T–13 probably was due to the segregation of factors for pathogenicity in heterozygous spores or to mutation.

It would seem from these results with T–11 that a race may appear to be essentially pure throughout a series of tests and then break up into two or more races.

SUMMARY

Five new races of *Tilletia tritici* and *T. levis* are described and numbered, thus bringing the total number of known races to 24, 14 of the former and 10 of the latter species.

Some of the races of *Tilletia tritici* and *T. levis* are distinguishable by one or more of the following criteria, other than pathogenicity: bunt-ball size, shape, and water absorptive properties; chlamydospore size, echinulation, and color; capacity to stunt the hosts and to stimulate excessive tillering of infected plants; incomplete smutting and capacity to intensify pigmentation of the glumes.

Race T–11 gave rise to T–13, one of the new races, thus indicating that some races are pathogenically unstable. Most of the races, however, proved highly stable in the tests on winter wheats at Pullman, Wash.

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PHYSIOLOGICAL STUDIES ON TWO SPECIES OF DIPLODIA PARASITIC ON CORN[1]

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INTRODUCTION

The two species of the genus *Diplodia* that occur most commonly on corn, *Zea mays* L., are *D. zeae* (Schw.) Lév. and *D. macrospora* Earle. Macroscopically, these fungi are very similar; but there is a marked difference in the size of the pycnospores, the spores of *D. macrospora* measuring 70–80 × 6–8 μ, while those of *D. zeae* measure 25–33 × 5–6 μ. In contrast to the morphological resemblance of the fungi and the similarity in appearance of decayed corn ears, the organisms exhibit distinct physiological differences. In all their contrasting characters, at least in this country, *D. zeae* seems the more vigorous. The two fungi also differ greatly in geographical range. *D. zeae* apparently occurs wherever corn is grown and causes considerable damage to the crop as a stalk and ear rot and at times as a seedling blight. In the United States *D. macrospora* has been reported chiefly from the southeastern states of Florida, Louisiana, Alabama, North Carolina, South Carolina, and Tennessee (10). *D. macrospora* also has been found in Brazil, Argentine, and Africa (7, 10, 20).

Both Miss Johann (7) and Miss Kinsel (8) have reported that *Diplodia zeae* grows more vigorously on synthetic media than does *D. macrospora*. Stevens (18) found that *D. zeae* grew faster in culture at all temperatures permitting growth of the two fungi. Apparently, *D. macrospora* cannot compete with *D. zeae* when the two are growing upon the same substrate, for Hoppe (6) was unable to recover *D. macrospora* from ears of corn infected with both organisms.

As a result of studies upon various fungi growing in synthetic nutrient media, Miss Kinsel (8) announced that *Diplodia macrospora*, under the conditions of her experiment, could not utilize monosaccharides as a source of carbon, but grew readily when supplied with di- or polysaccharides. Stevens and Larsh (19), using 24 isolates of *D. macrospora* from the southeastern United States and Argentine, found that the characteristic noted by Miss Kinsel was common to all these isolates, regardless of the source of nitrogen supplied. In contrast, Miss Kinsel secured an excellent growth of *D. zeae* on media containing monosaccharides, as well as on solutions containing more complex carbohydrates. More recently, Margolin (12) has indicated that *D. macrospora* makes only a sparse growth when supplied

[1] Material in this paper was taken from a thesis presented to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in botany.

[2] Hearty thanks are due Professor Neil E. Stevens under whom this work was done for his many helpful suggestions during the course of these investigations and in the preparation of this article.

with either sucrose or glucose, unless a needed growth substance is present in the medium. H. J. Fuller, in a paper not yet published, found that *D. macrospora* and *D. zeae* form amylase, invertase, maltase, catalase, and peroxidase in essentially the same quantities. It also has been reported that neither fungus is stimulated by thiamin (12, 15).

In order to obtain further information on the physiology of these fungi and, if possible, reconcile the somewhat conflicting results reported by earlier workers, further culture studies were undertaken with special reference to carbohydrate metabolism.

STUDIES ON NUTRIENT MEDIA

The basic medium used in the work described herein was the same as that employed by Stevens and Larsh, since these investigators found that *Diplodia macrospora* will grow more readily on this medium than on the more concentrated solution used by Miss Kinsel. The materials contained in this basic medium were: $MgSO_4$ 0.25 g., KH_2PO_4 0.3 g., KNO_3 2.0 g., carbohydrate 30.0 g., and distilled water to make one liter. The original source of the inoculum used in these studies was obtained from diseased ears of corn kindly furnished by P. E. Hoppe.

Since it was known that *Diplodia zeae* could utilize simple sugars, as well as the more complex carbohydrates, a check culture of *D. zeae* was always used in each lot of modified medium tested for its ability to support a mycelial growth of *D. macrospora*. The fungi were grown at room temperature in 125 ml. Pyrex flasks containing 50 ml. of medium that had been autoclaved at 15 pounds' pressure for 20 minutes. In most instances 10 flasks of each medium were inoculated.

Bits of dormant mycelium from old cultures were used as inoculum. It should be stressed that all inoculum used was brown and showed no evident signs of growth. Leonian and Lilly (11) have reported that the inoculum had no effect upon the growth of the colony of *Phycomyces blakesleeanus* in culture, but, as will be explained below, our experiments indicated that this does not hold for *Diplodia macrospora*.

Varied Sources of Inoculum. Inoculum taken from an old dormant culture of *Diplodia macrospora* invariably gave no significant growth on the basic medium containing only a simple sugar as a source of carbohydrate. A similar bit of such inoculum placed in a culture containing only whole oats produced a luxuriant mycelium. Experiment showed that a wisp of the mycelium from such a vigorously growing oat culture developed a good growth when placed in dextrose medium. It was found that hyphae from corn meal agar plates inoculated with kernels of corn infected with *D. macrospora* likewise gave rise to a sparse mycelium when placed in dextrose medium.

Removal of Trace Elements. The importance of minute quantities of heavy metals in the nutrition of fungi and the possible role such elements may play as limiting factors in the growth of fungi have been properly em-

phasized by Steinberg (17). Trace elements apparently are not responsible for the inability of *Diplodia macrospora* to utilize simple sugars, for, when these trace elements were removed from the nutrient solutions by the calcium carbonate method of Steinberg (16), slightly revised at his suggestion, it was found that *D. macrospora* made no growth on dextrose media so treated, but grew well on treated sucrose solutions. *D. zeae* formed abundant mycelium in solutions of both sugars whether treated or not.

Effects of Char Purification. The passage of a sucrose solution through finely ground animal char gave a complete inactivation of the sugar for growth of *Diplodia macrospora.* Purification of sucrose with Norite likewise effectively prevented development of this fungus. Other tests showed that a combination treatment of a sucrose solution with both Norite and calcium carbonate did not prevent the formation of mycelium by *D. zeae.*

Effects of Invertase Solution. A sucrose culture upon which *Diplodia macrospora* is growing gives a strong test for reducing sugars when treated with Benedict's solution. Although it is known that sucrose yields equal parts of dextrose and levulose when hydrolyzed, experiment confirmed Miss Kinsel's observation that no significant growth of *D. macrospora* resulted when the fungus was supplied with equal parts of commercial dextrose and levulose as a source of carbohydrate.

In an attempt to determine the ability of completely inverted sucrose to support growth of *Diplodia macrospora,* 10 g. of sucrose were dissolved in 330 ml. of distilled water, a 10 ml. ampoule of "Difco" invertase solution added, and the solution allowed to stand for several hours at room temperature. At the end of this time an examination of the solution by means of the polarimeter showed the sucrose to be completely inverted. After the inversion of the sugar, minerals were added and the medium flasked and autoclaved. *D. macrospora* grew well on this solution as did *D. zeae.*

The addition of an invertase solution to a glucose medium likewise made possible a heavy growth of *Diplodia macrospora,* which practically equalled the development of the fungus upon the sucrose invertase mixture. The average weight of 5 mats of *D. macrospora* grown on inverted sucrose was 470 mg.; the average of 5 mats from a dextrose-invertase solution was 450 mg. A check medium containing no other carbohydrate than that that the invertase may have carried gave only an extremely sparse growth of the two fungi.

Growth of Diplodia macrospora on Media Staled by Other Fungi. A chance observation of a flask containing *D. macrospora* on a sucrose solution that had become contaminated with an unidentified fungus showed that the growth of *D. macrospora* was much better in the contaminated flask than in the other flasks of the set. Following this lead, dextrose medium was staled by allowing *D. zeae* to grow on it for several weeks until the solution became amber or light brown. This staled medium was filtered to remove the mycelium of *D. zeae* and the filtrate was flasked and again autoclaved. *D. macrospora* made a very satisfactory growth on this staled

dextrose solution. These results are similar to those reported by Allison and his coworkers in their studies on coenzyme R (1, 5).

On media containing other simple sugars, such as levulose, sorbose, galactose, rhamnose, mannite, and also glycerol, *Diploidia macrospora* made little or no significant growth, as Miss Kinsel (8) reported. *D. zeae,* however, developed satisfactorily on all of these substances. After being staled by *D. zeae* for 4 or 5 weeks, media containing these various compounds all supported a good growth of *D. macrospora.* A little staled medium added to an unstaled dextrose solution also stimulated a good development of *D. macrospora.*

Growth-promoting Substances. By using a method corresponding to that employed by Buston and Pramanik (2) to extract a growth factor from lentils, a substance was obtained from corn grains and from whole oats that exerted a definite stimulating effect upon the growth of *Diplodia macrospora.* When 2 g. of either of these grain extracts was added to a half liter of dextrose medium and the solution inoculated with *D. macrospora,* the fungus made a vigorous growth. The addition of greater quantities of the substances provided no more growth stimulus than did the smaller amount, and, in the case of the oat extract, the larger amounts inhibited mycelial growth.

Green cornstalks, stock molasses, sugar beets, and a dextrose solution staled by *Diplodia zeae,* also provided an alcohol-extractable growth substance. It is significant to note, however, that an extract derived from sucrose media staled by *D. macrospora* was not active.

A growth factor for *Diplodia macrospora* was likewise obtained by carefully removing vigorously growing mycelium of *D. macrospora* from the surface of whole-oat cultures and macerating these hyphae in a little water. The resulting liquid was then filtered, and its growth-promoting properties tested by adding a little to a dextrose medium. On such media *D. macrospora* formed a heavy growth.

Through the courtesy of Leon Leonian, a small quantity of a biotin-like substance extracted from dung was made available for testing. This material enabled *Diplodia macrospora* to form a heavy mycelium on a sucrose medium purified with Norite. Later a commercial biotin concentrate, standardized at the laboratory by the assay method of Snell, Eakin, and Williams (14), was tested. Added to a dextrose medium at the rate of 0.02 gamma per 50 ml. flask, this substance produced mats of *D. macrospora* averaging 115.5 mg. after 4 weeks. A concentration of 0.05 gamma per flask was somewhat less effective.

Ineffective Growth Substances. In an attempt to ascertain the identity of this substance required for growth of *Diplodia macrospora,* a number of known compounds were added to dextrose media. All of these compounds had been reported as growth-promoting for various fungi. Nicotinic acid and vitamin C were tested in two dilutions, 0.1 mg. and 0.5 mg. per 50 ml. flask, but induced no growth of *D. macrospora.*

Other materials tested were C.P. i-inositol, 0.2 g. per liter; vitamin B_6 kindly furnished by Merck and Co., 0.2 mg. per liter; vitamin B_1 0.2 mg. per liter; and a combination of 40 mg. of vitamin C. and 0.2 mg. of vitamin B_1 per liter. None of these materials in the concentrations tested induced any mycelial formation whatsoever by *Diplodia macrospora* in dextrose media. Media containing 0.02 gamma of biotin per flask plus 10 mg. of i-inositol gave no better growth than the flasks containing only a like quantity of biotin; nor did the addition of 0.2 mg. per flask of para-amino benzoic acid to a medium containing biotin give any increased growth.

THE PROBABLE IDENTITY OF THE GROWTH FACTOR PRODUCED BY DIPLODIA ZEAE

As noted above, media containing simple sugars as a source of carbohydrate will support a satisfactory growth of *Diplodia macrospora* only if a suitable growth factor be added. Similar media, after being staled for several weeks by *D. zeae*, will support a good growth of *D. macrospora*.

Since it was known that a commercial preparation containing biotin enabled *Diplodia macrospora* to utilize dextrose, an effort was made to determine whether the substance excreted by *D. zeae* may be identical with biotin. A concentrate obtained from dextrose media staled by *D. zeae* supported a good mycelial formation of *D. macrospora* when added to dextrose media in small amounts. For this reason the concentrate was subjected to a number of the physical and chemical treatments by which biotin, coenzyme R, and vitamin H, three growth factors now considered to be identical (4), are tested.

As a result of these tests the growth factor elaborated by *Diplodia zeae* was found to possess a number of the characteristics exhibited by biotin obtained from egg yolks by Kögl and Tönnis (9).

It is thermostable, soluble in water and alcohol, but scarcely soluble in ether, petroleum ether, or chloroform. The substance is not destroyed by refluxing with acidulated methanol, is dialyzable, adsorbed by char, and is resistant to the action of strong acids and alkalies. In addition it is completely inactivated by nitrous acid.

Two additional tests also were made upon the concentrate containing the growth factor. Following a report (3) that a constituent of raw powdered egg albumen will inactivate biotin *in vitro*, the effects of egg white were determined. A second test concerned the ability of the concentrate to stimulate the growth of *Saccharomyces cerevisiae*, an organism believed to require biotin for growth.

The whites of fresh eggs were used in testing the relative effects of raw and cooked albumen upon the growth principle in the concentrate. In the first part of this experiment the white of an egg and 10 ml. of a solution of the concentrate were added to a half liter of distilled water and the mixture autoclaved for 20 minutes. The addition of dextrose and the required minerals was made after the initial heating, and the medium was then flasked and re-autoclaved. Both *Diplodia zeae* and *D. macrospora* showed an excellent development on this medium containing the cooked white of egg.

The experiment was completed by aseptically adding the raw white of an egg to a large flask containing a half liter of dextrose medium that had been autoclaved and cooled. The flask was shaken vigorously to break up the albumen and the solution was then poured into small flasks that had been previously sterilized. *D. macrospora* made no growth in media containing the raw white of egg if no contamination appeared. In one instance where the egg was apparently already contaminated by bacteria, *D. macrospora* made a fair development after 2 or 3 weeks. The raw white of egg also reduced to a marked degree the growth of *D. zeae*.

When supplied with Domino Cube sucrose in the "B" solution used by Robbins and Schmidt (13) for the growth of *Ashbya nematospora*, a culture of *Saccharomyces cerevisiae* furnished by Leon Leonian made only a slight growth. On a similar medium, to which a little of the concentrate had been added, the yeast grew very satisfactorily.

GROWTH FACTOR PRESENT IN CERTAIN CARBOHYDRATES

The fact that *Diplodia macrospora* will grow readily on simple sugars, if supplied with suitable growth substances, suggested that the results reported by Miss Kinsel (8) and by Stevens and Larsh (19) might be traceable to impurities, present in the complex carbohydrates, removed from the monosaccharides in further processing. It also seemed reasonable to believe that very pure di- or polysaccharides also might be lacking in these substances needed for the growth of the fungus. Several tests of various carbohydrates were undertaken in an attempt to determine the facts.

Preparation of Dextrose. Since *Diplodia macrospora* was observed to grow when supplied with corn starch but did not develop on dextrose, obtained commercially by the hydrolysis of starch, it seemed desirable to study the steps in the process of the conversion of starch to dextrose. Through the courtesy of J. Paul Bishop of the Corn Products Refining Co. of Argo, Illinois, samples of hydrolyzed starch from various stages of the processing by which starch is converted to dextrose were made available. The samples tested for their ability to support growth of the two *Diplodias* were as follows: (1) starch hydrolysate neutralized with sodium carbonate; (2) the neutralized solution with fats removed by centrifuging and the centrifugal liquor filtered and passed over char once; (3) decolorized and concentrated liquor passed over char twice and ready for the crystallizer, (4) Hydrol, the second mother liquor removed from the sugar crystals by centrifuging; (5) special Hydrol, the second mother liquor specially treated to remove inorganic salts and all kinds of acids, (6) Alpha dextrose hydrate, obtained by crystallization; and (7) fatty material removed during the first centrifuging of the hydrolyzed starch solution.

These samples were evaporated to about 70 per cent dryness at the Corn Products laboratory and consequently, with the exception of the dextrose and fatty material, were used at the rate of about 40 g. per liter of medium. The results obtained are listed in the following table.

TABLE 1. *Growth record of Diplodia macrospora and D. zeae cultured for 7 weeks at room temperature*

Sample	*D. macrospora*		*D. zeae*
	No. of flasks	Av. net wt.	(1 flask)
Starch hydrolysate neutralized	9	109.4 mg.	Good
Liquor passed over char once	9	240.0	Good
Liquor ready for crystallizer	8	93.7	Good
Hydrol	9	294.4	Good
Dextrose	9	Negligible	Fair
Dextrose plus 10 g. of Hydrol per liter	9	197.2	Fair
Dextrose plus 10 g. of fatty centrifugate per liter	9	Negligible	Poor
Special Hydrol (40 g. per l.)	7	90.0	Fair
Special Hydrol (60 g. per l.)	7	97.1	Fair

Inulin. In the tests of inulin, two grades were used; Pfanstiehl C.P. and Pfanstiehl "Practical." *Diplodia macrospora* made a sparse growth when furnished with these samples of inulin in culture, averaging only 31.1 mg. per flask on the C.P. grade and 35.8 mg. on the "Practical." However, it should be noted that on another sample of C.P. inulin *D. macrospora* made a much better development. Unfortunately, no weight determinations were made of the fungus when grown on this particular sample. Inulin hydrolyzed by the addition of a little hydrochloric acid also supported a fair growth of *D. macrospora.* The best development of the fungus occurred when a little growth substance was added to the medium. *D. zeae* grew fairly well on both grades of inulin.

Maltose. When *Diplodia macrospora* was inoculated into media containing maltose the results obtained varied to a marked degree according to the grade of the sugar used. On Pfanstiehl C.P. maltose the fungus averaged only about 30 mg. per flask,[3] but, when grown in a medium containing a brown colored technical grade, a luxuriant mycelium was formed, which averaged 427.1 mg. per flask. That impurities were responsible for the stimulation of growth seems evident because passage of the sugar through finely ground animal char prevented any growth of *D. macrospora.*

Sucrose. Development also varied according to the source of the sucrose used. Sucrose samples from 5 sources were tested for their ability to support growth of *Diplodia macrospora.* These included C.P. sugar from two chemical companies, two commercial brands of cane sugar, and a sample of bulk beet sugar. The comparative results obtained are listed below.

TABLE 2. *Growth record of Diplodia macrospora and D. zeae cultured for 4 weeks at room temperature on various samples of sucrose*

Brand of sucrose	*D. macrospora*	*D. zeae*
	(Av. of 9 flasks)	(1 Flask only)
Bakers "Analyzed" C.P.	41.1 mg.	Satisfactory
Coleman & Bell C.P. Saccharose	90.0	"
C&H "Pure Cane" from 2 lb. box	48.1	"
Domino Cube	27.8	"
C&W Beet Sugar from 10 lb. bag	Very sparse	"

[3] Average of only 3 flasks.

Coleman and Bell's C.P. sugar was commonly used as a disaccharide in our cultures, and, as a rule, permitted even more satisfactory growth of *Diplodia macrospora* than that obtained in the above comparison. The fungus consistently failed to make any significant growth on the Domino Cube sugar, unless supplied with growth factor of the biotin type. This was true also of the single sample of beet sugar tested.

DISCUSSION

The results obtained from these studies indicate that one of the fundamental physiological differences between *Diplodia zeae* and *D. macrospora* is the inability of *D. macrospora* to elaborate a growth substance required by both fungi in their metabolism. The fact that this fungus did not develop on unstaled media, containing only dextrose as a carbohydrate, but grew very well in similar media after being staled by *D. zeae*, regardless of whether or not trace elements were removed, was direct evidence that the growth of *D. macrospora* was induced by an organic substance secreted by *D. zeae*.

The occurrence of the growth factor for *Diplodia macrospora* in corn and oat grains, sugar beets, molasses, and green cornstalks indicates that this growth substance is not uncommon. The stimulating effect of invertase solution may be explained as the effect of a growth substance present as an impurity in the solution.

When grown on whole oats, *Diplodia macrospora* obtained an abundance of the required metabolite from the substrate. This was evidenced by the vigorous hyphal growth made by the fungus and by the stimulating effect that an aqueous filtrate, derived by crushing these hyphae of *D. macrospora*, exerted upon the development of the fungus in a dextrose medium. The presence of accumulated growth factor in the hyphae would account for the growth of *D. macrospora* in dextrose media when the inoculum used was bits of mycelium from vigorously growing cultures. For this reason only inoculum from dormant mycelia was used throughout the course of these investigations. The growth principle, carried over by bits of mycelium growing vigorously on agar, would seem to account for the development of *D. macrospora* obtained by Margolin when the fungus was supplied with dextrose. This appears even more probable, since a biotin-like material apparently was added to the agar on which the *D. macrospora*, used as inoculum, was grown.

The results of the various tests to which the concentrate obtained from dextrose media staled by *Diplodia zeae* was subjected indicate that in all probability biotin is the growth factor that *D. zeae* elaborates, and is a substance that both *D. zeae* and *D. macrospora* require in their metabolism. One of the most convincing pieces of evidence that *D. zeae* synthesizes biotin was furnished by the stimulating effect that the concentrate had upon the growth of *Saccharomyces cerevisiae*, a yeast believed to need biotin for growth.

As Margolin has suggested, the reason that Miss Kinsel (8) and also Stevens and Larsh (19) obtained growth of *Diplodia macrospora* only when the fungus was supplied with complex carbohydrates may be explained as the effect of a needed growth factor present as a contaminant in many di- and polysaccharides. Why the growth substance is not present in simple sugars, such as dextrose, which is obtained from a contaminated polysaccharide, is not so apparent. The tests run on the samples of processed starch that was being converted to dextrose indicated that sufficient growth substance to induce development of *D. macrospora* was present in the liquor placed in the crystallizer. Judging from the growth of the fungus, the growth factor was present also in Hydrol, the mother liquor removed by centrifuging from the dextrose crystals after the process of crystallization was completed; yet *D. macrospora* made no significant development on medium containing dextrose, unless a growth factor was supplied.

In the process of refining cane sugar a similar method for removing the molasses from the crystals by centrifuging is followed. Nevertheless, the growth of *Diplodia macrospora* indicates that an appreciable quantity of a needed growth factor may remain in association with the crystals of cane sugar. At present the only reason the writer can suggest for this fact is that the larger carbohydrate molecules may have an affinity for the growth factor, which the smaller molecules of dextrose do not possess. More study is needed before a definite explanation of this phenomenon can be given.

The very high purity of some commercial sucrose, at least insofar as the presence of growth factor is concerned, was indicated by the comparative growth of *Diplodia macrospora* obtained on these sugars and on the C.P. grades. The slight growth induced by Domino Cube sugar is apparently due to the extreme purity of the product. L. A. Wills of the American Sugar Refining Co., which produces Domino sugar, explained in private correspondence that, although ordinary commercial grades of cane sugar have a high purity of approximately 99.5 per cent, the special method of refining used in producing Domino Cube sugar gives a product that is at least 99.95 per cent pure.

The writer has no explanation of the high purity of beet sugar, as relates to the presence of growth factor, except to point out that Snell, Eakin, and Williams (14) found that sugar beet molasses in some instances contains very much less biotin than does cane sugar molasses. This may indicate that sugar-beet roots naturally contain less biotin than do cane stalks.

White has stated (21) that the source of sucrose has no significant effect upon the growth of excised tomato roots in culture, but these experiments do not warrant a similar conclusion for the growth of *Diplodia macrospora*. The high purity of the sucrose (Bakers "Analyzed" C.P.), employed by Margolin, probably accounts for the sparse growth he obtained when he attempted to culture *D. macrospora* on sucrose.

Our results emphasize that the possibility of introducing growth factors, present as impurities in the carbohydrates employed, should be borne in

mind by all investigators who grow organisms in synthetic solutions. That these growth factors are more often present in complex carbohydrates than in hexose sugars is abundantly borne out by these studies. Steinberg (17) has suggested that "repetition of these tests with hydrolyzed polysaccharides would appear desirable to determine whether this effect is due to chemical structure or to the greater difficulty in purification of the polysaccharides" in those instances where it has been found that certain fungi develop more readily on polysaccharides than on simpler compounds. From the results of these studies with *Diplodia macrospora* it now appears that the more complex carbohydrates are more difficult to purify since, with the exception of one sample of dextrose, none of the monosaccharides tested was of C.P. grade. In contrast, C.P. sucrose was commonly used when a disaccharide was required.

SUMMARY

The results of this series of physiological studies on *Diplodia macrospora* and *D. zeae* having special reference to the utilization of various carbohydrates may be summarized as follows:

The source of inoculum of *D. macrospora* was found to be correlated with the ability of this fungus to grow on simple sugars supplied in the basic nutrient medium.

A substance that enabled *D. macrospora* to develop on media containing simple sugars was obtained from corn, oats, sugar beets, molasses, hyphae of *D. macrospora* from vigorously growing cultures, and a dextrose medium staled by *D. zeae*. An invertase solution and a commercial biotin concentrate were also effective in promoting growth.

The growth substance elaborated by *D. zeae* and present in a concentrate of dextrose media staled by this fungus was observed to possess many of the properties of biotin. It is believed to be biotin or a closely related compound.

Tests made on samples from various stages of the process by which starch is converted to dextrose indicated that the growth factor present in starch is not present in dextrose in significant amounts, but remains in the mother liquor removed from the dextrose after crystallization.

The growth of *D. macrospora* was definitely influenced by the source of the maltose and sucrose supplied in the culture media.

The results obtained indicate that *D. macrospora* can utilize complex carbohydrates in many instances only because they contain as an impurity a growth factor required by this fungus. This growth factor apparently is removed from simple sugars during the manufacturing processes.

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THREE BACTERIAL PLANT PATHOGENS: PHYTOMONAS CARYOPHYLLI SP. N., PHYTOMONAS ALLIICOLA SP. N., AND PHYTOMONAS MANIHOTIS (ARTHAUD-BERTHET ET BONDAR) VIEGAS

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The three bacteria described in this article have little in common except that they are plant pathogens. Two are new species, while the third has received no adequate description, although the name has appeared in literature for over 25 years.

In the following investigation, isolates of all three species were run simultaneously and the methods used were the same. To avoid repetition the methods are given in the following paragraphs. In most instances these are well known and only need be mentioned.

In measuring the bacteria the Congo red negative stain was employed, while Plimmer and Paine's modification of the Casares-Gil's stain was used for determining possession and position of flagella. The Ehrlich-Böhme method was employed for the determination of indol in tryptone broth, while strips of filter paper impregnated with lead acetate were suspended over the same medium for the determination of hydrogen sulphide production. M. P. Starr made the lipase test, using his own method, which has been published recently (17). The synthetic nitrate medium listed in the Manual of Methods (16) was employed in the nitrate studies.

In determining the substances that can be used for an energy and carbon source the following method was employed. The synthetic basal medium as recommended in the Manual of Methods (16) was used, and was tubed and autoclaved at the usual pressure and time. The carbon sources to be tested, with the exception of the salts of the organic acids, were prepared in 10 per cent aqueous solutions, sterilized by filtration, and then added to the tubes of basal media, so that a 1 per cent solution was obtained. Sodium salts of the organic acids were added directly to the basal media, making up 0.15 per cent of the solution, and autoclaved together. Ammonium lactate was used instead of the sodium salt, since this latter was not on hand. For the determination of starch hydrolysis both the starch-agar iodine method and starch broth method were used.

CARNATION PATHOGEN

Early in 1940 L. K. Jones sent the writer a number of wilted and dead carnation plants. In an accompanying letter he stated that the disease had been found in Washington State, where it was severe in one of the greenhouses, and that it was caused by a bacterium he had isolated and whose pathogenicity he had proved. In the correspondence that followed it was agreed that he should attend to the pathological phases of the disease and

the writer the etiological phases—namely the description of the pathogen. Since then, Jones has published a description of the symptoms of this disease (11). In brief, the affected plants wilt, lose their color, and become dry, while the roots entirely disintegrate.

Accompanying the first specimens was also a culture of the bacterial pathogen; and later, when a great many diseased plants were received, 20 isolates were made. In most cases these were obtained from the interior of the stalks a little aboveground. Below ground, the roots were well rotted and many secondary organisms were present. These cultures were all from specimens sent from the West Coast, but it is possible that the disease is of greater distribution. In appearance it is similar to the fusarium wilt of this plant and easily might be mistaken for it. J. M. Bickerton told the writer that he had obtained at times specimens of diseased carnations that he took to be the fusarium wilt, but in which he was unable to find the fungus.

While 20 isolates were on hand at the beginning of the work only 12 were used in the various tests for determining the characteristics of the pathogen. The reduction in number was due to the death of 8 cultures that were being grown on an unfavorable medium. This medium was the ordinary beef-extract-peptone agar, and, although the cultures grew readily on it for a few days, they did not remain viable. Potato-dextrose agar was found to be a more satisfactory medium.

The pathogenicity of all the isolates was proved by inoculation into carnation plants, and after symptoms of the disease appeared the pathogens were reisolated. The carnation variety, Virginia, was used as the host plant in most experiments. Infection readily can be obtained by scraping a small mass of bacteria from a colony on agar onto a sharply pointed scalpel and, with this contaminated instrument, piercing the carnation stalk a little above the ground. Definite symptoms appear in approximately 4 weeks. Healthy carnation plants transplanted to pots that previously had disease in them or in which suspensions of the pathogen had been poured may contract the disease. Under these conditions the incubation period may be 2 months or more.

While no attempt has been made to determine the host range of the pathogen, inoculations were made on 2 plants of *Dianthus barbatus* L., and 1 plant each of *D. plumarius* L. and *D. allwoodii*, a horticultural designation of a race of hardy pinks. These were full-grown plants that happened to be in the greenhouse at the time the work was in progress. Only *D. plumarius* remained healthy.

All the isolates employed in describing the pathogen, were from single colonies, and dilution plates were made 3 to 4 times with each to insure purity. In the following tests with the 12 isolates, a uniformity of reactions existed.

Morphology. The pathogen is a short, slender rod, at times slightly curved. In 48 hour-old cultures on potato-dextrose agar at 27° C. the size of the cells was 1.84 μ (1.05 to 3.18 μ) by 0.56 μ (0.35–0.95 μ). The organism is actively motile by 1 to several flagella, frequently at both ends. It is Gram-negative.

Cultural Characteristics. Colonies, 4 to 5 days old on potato-dextrose agar are 3 to 4 mm. in diameter, circular, smooth, glistening, with edges entire. The consistency is butyrous. The color is a tan to a gray-mauve. This peculiar appearance is caused by a whitish film over the darker brown cells in the interior of the colony. Later, the film may disappear, and the colony becomes a deeper brown. On beef-extract-peptone agar the growth of the pathogen is similar to the above description, but the consistency of the colony is very firm and hard, so that it is difficult to remove portions of it. Beef-extract-peptone bouillon (pH 6.6) becomes very turbid in 24 hours at 27° C. to 36° C. A whitish sediment is formed. In litmus milk, growth is slow, the medium becomes slightly blue in 1 week, and a slight reduction of litmus begins at the bottom and advances about half way up the tube. In 6 weeks the color is a slate-blue with a dirty-white sediment. There is no clearing. In shake cultures with beef-extract, peptone agar plus 0.5 per cent dextrose used as a medium, colonies appear on the surface or only slightly below, showing that the pathogen is an aerobe. In Clara's medium (4) there is good growth but no green fluorescent pigment is produced.

The optimum temperature for growth is about 30 to 33° C. Maximum 46° and minimum 5° or less. The pathogen has an exceptionally high temperature range.

Biochemical Reactions. Growth in gelatin stabs is moderate and liquefaction begins only after 3 to 4 weeks, and proceeds very slowly. The liquefied medium is of oily consistency. Growth in tryptone broth is excellent, but indol is not formed nor is hydrogen sulphide. Asparagine, KNO_3 and $NH_4H_2PO_4$ can be utilized for nitrogen. Nitrates are reduced to nitrites, which soon disappear with the formation of ammonia and a gas. This reaction might possibly explain the destructive rot of infected tissue. Lipase is produced.

The carnation pathogen proved to be extremely saccharolytic. The following carbon sources were utilized: l-arabinose, d-xylose, rhamnose, dextrose, d-galactose, levulose (d-fructose), d-lactose, maltose and sucrose; glycerol, mannitol, and salicin, with a decrease in pH; and the sodium salts of the following acids, acetic, citric, formic, hippuric, lactic, maleic, malic, succinic, and tartaric with an increase in pH. Starch was not hydrolysed.

The above description of the bacterial pathogen and the type of disease it produces on the carnation plant, leaves little doubt that it is distinct from the disease caused by *Phytomonas woodsii* (E. F. Smith) Bergey *et al.* The latter is the only bacterial disease of carnations, as far as the writer is aware, that previously has been reported on this floral crop. *Bacterium dianthi* Arthur and Bolley now is considered a saprophyte. The description of the new carnation pathogen is so distinct in appearance and in action in media that it must be considered new. The name **Phytomonas caryophylli** n. sp. is proposed. Certain characteristics place it in the *Pseudomonas* group, as the characters of curved rods with polar flagella, the action on milk and asparagine, the use of formates and the lack of H_2S production and of starch hydrolysis.

ONION PATHOGEN

A number of bacteria have been reported in literature as causing or at least associating with rots of onion bulbs. *Bacterium allium* Griffith (9) was possibly the first to be described as isolated from rotting onion bulbs, but Griffith, who conducted the investigation, makes no claims that the species is pathogenic. The organism was evidently one of the green-fluorescent saprophytes. In 1906, Delacroix (5) isolated a bacterium from rotting onions and proved its pathogenicity. He named the pathogen *Bacillus cepivorus* n. sp., but gave an incomplete description of the organism, so that it is difficult to determine with what species he was working. Giampietro (8) claims to have found that *Bacterium coli* produces a rot of onions, and he further states that *B. cepivorus* and *Bact. coli* are identical. It is possible that both these men were working with *Erwinia carotovora* (Jones) Holland.

While the name *B. cepivorus* still appears in literature, especially from Europe, as far as the writer is aware no further work has been done on this species or the disease it produces. Numerous reports are on record that *E. carotovora* causes a soft rot of onions and several investigations from Jones (12) have proved its pathogenicity on this crop. Townsend (20) obtained a rot on sliced onion bulb with *Bacillus aroideae* when he described this species, and, later, Takomoto (18) found the same organism causing a rot of onions in Japan. *Bacillus croci* Mizusawa (13), a probable synonym of this pathogen, also has been reported on onion. Edelsztein-Kosowa (7) in Poland described a bulb rot of onion caused by a new species of bacterium, which he named *Bacillus cepae*. This pathogen is a spore former with peritrichic flagella, producing gas in carbohydrate media, and rapidly hydrolyzing starch. These characters readily differentiate it from the other pathogens.

Okabe (14) working with *Bacterium formosanum* Okabe, which the writer considers a synonym of *Phytomonas chicorii* Swingle, was able to obtain infection when onions were inoculated with this organism. Another synonym of *Phyt. chicorii* is *Phyt. endiviae* (Kotte) Clara (4). A culture of this latter bacterium was received several years ago from Kotte and recently artificial inoculations were made on onion bulbs. A definite light-brownish rot was obtained.

A number of writers have reported bacterial rots of onions without giving a name to the pathogen. The most interesting and extensive article of this type was in 1899 when Stewart (18) carefully described a rot of onion bulbs occurring in New York State, and published an excellent picture of a diseased specimen. While he was certain that the disease was bacterial he did not isolate or describe the organism. It has been thought by certain investigators that the cause of the disease reported by Stewart was *E. carotovora* but the work presented in this article throws some doubt on this point.

During the last 10 years or so, at infrequent intervals, rotting onion bulbs have been sent to the Department of Plant Pathology, Cornell University, for determination. In many instances certain inner scales of these diseased bulbs have been watersoaked and soft. In appearance the disease was not unlike a frost injury, but the rot did not extend horizontally into the adjacent scale. In early stages of the disease the bulb may appear sound on the outside, but, on being cut open, one or two of the scales may be found to be infected. In advanced stages the entire bulb may rot. Microscopic examination of even the advancing margins of the rot show they are full of bacteria.

It was thought at first, on examining such specimens, that the disease was caused by *Erwinia carotovora*. In the fall of 1936, however, attempts made to isolate *E. carotovora* from these rotting onions met with failure. In most cases, however, dirty white bacterial colonies appeared on the dilution plates and were characterized by coloring beef-extract peptone agar a deep-brown. The organism, when inoculated into onion bulbs, caused a rot, as described above. In 1939, the organism was again isolated from rotting onions. It

appeared from this second instance that the bacteria were of more than casual occurrence and, since the symptoms of the disease agreed so closely with Stewart's disease, they probably were of long standing.

Just how and when infection takes place to cause this bulb rot is not known. It probably is at harvest time, or slightly before, since young growing onion leaves are but slightly susceptible to the bacterium and appear to outgrow the disease. Mature bulbs are very susceptible and may rot completely within 10 days at room temperature. No rot has been found at 6° C. upon artificially inoculated bulbs.

Inoculations were made on a number of plants with this onion organism with the following results. The bacteria are able to produce a brownish rot on carrots, which is not so soft as that caused by *Erwinia carotovora;* a black but not extensive rot on paper-white narcissus bulbs; and a slight dry rot on tulip bulbs and on iris rhizomes. Potato tubers, bulbs of *Lilium speciosum,* and cabbage stalks were not infected.

The pathogen causing this onion rot is very readily isolated from the advancing margins of the lesions by making dilution plates with either potato-dextrose agar or beef-extract-peptone agar as the medium. The latter is preferable, since a dark coloration appears around the colonies of the pathogen on this medium, and, consequently, they can be recognized more easily.

In describing this bacterium 7 isolates were used, each from a separate onion bulb and each from a single colony. Each isolate was later plated in dilution plates 3 to 4 times and its pathogenicity proved. The only difference noted among the isolates was a difference in the intensity of the brown discoloration of the media. This coloration did not have the purplish tint characteristic of the tyrosine reaction.

Morphology. The onion pathogen is a rod with rounded ends. In a 24-hour-old culture on beef-extract-peptone agar grown at 27° C., the size of cells was 2.0 μ (1.05 to 2.80 μ) by 0.9 μ (0.7 to 1.4 μ). The bacteria are motile with 1 to several polar flagella, sometimes bipolar. They are Gram-negative.

Cultural Characters. Streak cultures on beef-extract-peptone agar are moderate in growth, white at first, but, later, dirty in appearance, edges of streak wavy, consistency viscid. Agar becomes a deep brown. On potato-dextrose agar growth is more abundant, mucoid, and white to a dirty-cream in appearance. Consistency is not viscid but rather watery. Medium not brown. In beef-extract-peptone bouillon, growth appears in 24 hours or less, becoming turbid with a slight pellicle. Browning of broth begins near surface and extends downward into the tube. In litmus milk, growth is at first slow but, by 5 days, litmus is reduced and milk cleared. Medium approximately neutral. In shake cultures with beef-extract-peptone agar plus 0.5 per cent dextrose, the colonies appear only at surface. The upper quarter of medium becomes cleared and a light yellow-green in appearance. Later the medium becomes brown. In Clara's solution (4), there is a light to cloudy growth with a white sediment, but no green fluorescent pigment.

The optimum temperature for growth is about 30° C., maximum 41° C., and minimum 5° C. or less.

Biochemical Reactions. Growth in gelatin stabs is good, liquefaction begins on the second day, and in 4 weeks half of medium is liquefied and brown. Growth in tryptone broth is good, but indol is not formed nor is hydrogen sulphide. Asparagine, KNO_3 and $NH_4H_2PO_4$ can be used for the nitrogen source. Nitrates are reduced to nitrites. The organism shows excellent lipolytic action. The following carbon sources utilized: l-arabinose, d-xylose, rhamnose, dextrose, d-galactose, levulose (d-fructose), d-lactose, maltose sucrose, glycerol, mannitol, and salicin produced growth with an acid reaction. Rhamnose, however, produced a very weak growth. The salts of acetic, citric, formic, hippuric, lactic, malic, succinic, and tartaric acids produced growth with an alkaline reaction. Maleic acid was not utilized, nor was starch hydrolysed.

Taxonomy. The numerous bulb rots of onions that have been described from time to time are very similar in symptoms, and, possibly, they could be differentiated only after a comparative study of the various diseases. The bacterial pathogens causing these diseases, however, are distinctly different and are readily separated. The pathogen under consideration in this article does not agree with any of the previously described bacterial pathogens affecting onions; it appears to be a pathogen heretofore unrecognized. Bacteriologically the onion pathogen is similar to *Phytomonas solanacearum* (E. L. Smith) Bergey *et al.* It retains its virulence in culture fairly well, however, which the latter species does not do. As far as the writer is aware *Phyt. solanacearum* has not been reported on onion, and the onion pathogen does not infect potato tubers. Possibly a still more closely related species is *Phytomonas gardeniae* Burkholder and Pirone. Here again the onion pathogen produces no infection on gardenia leaves, even under very favorable circumstances. For this reason it is considered a new species, and the following name is proposed—**Phytomonas alliicola** sp. n. The pathogen probably could be placed in the *Pseudomonas* group.

CASSAVA PATHOGEN

In the summer of 1939 Karla Longrée received some cuttings of Cassava (*Manihot utilissima* Pohl.) of the variety Cambaia from A. P. Viegas of São Paulo, Brazil. The cuttings were rooted in one of the greenhouses at Cornell University and, for a time, they grew vigorously. However, after a few weeks of growth about 10 of these plants began to show signs of a wilt. The lower leaves flagged, then eventually the upper leaves and finally the whole plant died. Upon a careful examination of such plants it was discovered that the vascular system was full of bacteria that, in many cases, had broken through the cells and even on to the surface of the stem, where they occurred in sticky drops.

In correspondence with Dr. Viegas he stated that the disease was generally recognized in Brazil and was of economic importance. Bacteriologically little was known concerning the cause, and he suggested that the writer study and describe the pathogen.

In a search through literature greatly aided by A. P. Viegas it was found that 3 bacterial diseases have been described on Cassava. Bondar (1) in 1912 was the first to report one on this crop and later in 1915 (2) gave a fuller account of the trouble. The disease was found in Brazil, and the host species he gives is *Manihot palmata.* From all appearances it was this disease that was on the Cassava cutting at Cornell University and this conclusion was substantiated by A. P. Viegas. Bondar attributed the cause of the disease to a bacterium, which, in his first article, he names *Bacillus manihotus* Arthaud-Berthet,[1] but with no description. Dr. Arthaud-Berthet was collaborating with him on the problem, although his name does not appear as co-author of the article. The second article also is signed by Bondar alone

[1] The hyphen in this name is not used consistently.

but a note on page 911 of this volume states that Sr. Arthaud-Berthet's name, accidently, was omitted. Here the pathogen is called *Bacillus manihot* sp. n., and a very inadequate description is given of it. Since then the name may be found in literature as *Bacillus manihotus* Arthaud and Berthet, which is incorrect. Recently A. P. Viegas informs me that in an article in O Campo 11: No. 132; 64, 1940, which I have not seen, Bondar uses the name *Bacillus manihoti* Berthet et Bondar. Viegas (21), however, has placed the pathogen in the genus *Phytomonas* and calls it *Phytomonas manihotus* (Arth. Berth. and Bondar) n. comb. In a letter Viegas states that the "u" in the specific name is a typographical error. It should be "i."[2]

Two other diseases of Cassava caused by bacteria also are reported in literature. Miss Schwarz (15), in 1926, proved that *Phytomonas solanacearum* (E. F. S.) Bergey *et al.* was capable of attacking this plant (*Manihot utilissima* Pohl.) and causing a slime disease. Since then, others have reported finding this disease. Hansford (10), in 1938, described a leaf spot and stem disease of Cassava from Uganda. No wilt is mentioned. The pathogen *Bacterium cassavae* sp. n. belongs to the genus *Erwinia*. These two latter diseases cannot be confused with the one under consideration.

The bacteria from the affected plants in the greenhouse at Cornell University were readily isolated in either beef-extract-peptone agar or potato-dextrose agar. They conformed to Bondar's description of *Bacillus manihotus* in that they were white, grew slowly, and were aerobic. The only other character Bondar mentions is that his species was Gram-positive. The recently isolated bacteria were Gram-negative, but, as the writer has stated at other times, the Gram stain is difficult to handle, and many bacteria described at one time as Gram-positive, are now known to be Gram-negative.

In describing the present Cassava organism, 8 isolates were used. These were selected from single colonies. Dilution plates were made 3 times and the resulting isolates tested for pathogenicity. All behaved similarly both pathologically and in culture.

Morphology. The pathogen is a very slender rod. A 24-hour-old culture on beef-extract-peptone agar at 27° C. yielded the following measurements for the cells, 2.17 μ (1.4 to 2.8 μ) by 0.6 μ (0.35 to 0.93 μ). The pathogen is Gram-negative and probably nonmotile. Excellent flagella stains were obtained on the other two pathogens discussed in this article, but in only one culture of the Cassava organism could one find a very limited number of cells with a single polar flagellum. These might have been contaminants, although, in one instance, Viegas informs me he also noted motile cells.

Cultural Characters. Streaks on beef-extract-peptone agar at 27° C. show up in about 48 hours, are raised, ivory-color, smooth, shiny, with edges entire. Their consistency is watery. On potato-dextrose agar, growth is more abundant, white to hyaline, and very mucoid. Beef-extract-peptone bouillon becomes cloudy with a white granular ring. In litmus milk, growth is good and a clearing and reduction of litmus takes place in 3 to 4 days at 27° C. With the return of color to the litmus the medium is purple. In shake cultures (beef-extract-peptone agar plus .5 per cent dextrose) most of the colonies were on or at the surface. Later a few appeared deeper in the medium. There is no growth in Clara's medium.

The optimum temperature for growth is approximately 30° C., maximum 38° C. and minimum 5° C.

[2] Since this present paper was accepted for publication Drummond and Hipólito's recent investigation (6) was brought to the writer's attention. They describe the Cassava wilt pathogen and use the name *Bacterium manihotus*.

Biochemical Reactions. Growth in gelatin stabs is good. Liquefaction begins on the 4th day, and, in 2 weeks, half the medium is liquefied. Growth in tryptone broth is good. Indol is not formed but hydrogen sulphide is. The organism cannot utilize nitrates or asparagine. Ammonium salts, however, are utilized for nitrogen. There is a doubtful lipolytic action.

Since the pathogen does not grow well in synthetic media, some difficulty was experienced in determining the carbon sources it could utilize. A very weak growth with a slight acid production was obtained with dextrose, d-galactose, levulose (d-fructose), d-xylose, maltose, and sucrose. Rhamnose, l-arabinose, d-lactose, glycerol, mannitol, and salicin gave no growth. In one series a better growth was obtained with levulose when .1 per cent of sodium thio-glycolate was added than when left out. The use of this material was not followed up, however. A good growth with an alkaline reaction was obtained with the salts of the organic acids, acetic, citric, malic, maleic and succinic. The salts of formic, hippuric, lactic, and tartaric were not utilized. Starch was not hydrolysed.

For the present this organism had best be placed in the genus *Phytomonas* and the following name used, *Phytomonas manihotis* (Art.-Berth. et Bondar) Viegas. Steps are being undertaken, however, to drop this genus and allocate its members to other genera. Where this species then would be placed has not been determined. It seems to be one of those intermediate types having relationships with the genus *Pseudomonas* and that small group of Gram-negative vascular parasites, as *Phytomonas albilineans* (Ashby) Magrou, *Phytomonas stewartii* (Erw. Smith) Bergey *et al.* and *Phytomonas tardicrescens* (McCulloch) Burkholder. Further work on its relationships to other bacteria is needed.

SUMMARY

A description is given of three bacterial pathogens. Two are new species, **Phytomonas caryophylli** sp. n., which causes a wilt and root rot of carnations, and **Phytomonas alliicola** sp. n., which causes a bulb rot of onions. The third description is of *Phytomonas manihotis* (Arthaud-Berthet et Bondar) Viegas, a bacterium causing a wilt of Cassava and of which no adequate description has been given hitherto.

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LEAF-SPOT DISEASE OF CULTIVATED SALSIFY

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In August, 1933, the writer found on Staten Island, New York, an apparently undescribed leaf spot of cultivated salsify (*Tragopogon porrifolius* L.), caused by a variety of *Stemphylium botryosum* Wallr. The lesions were confined to the foliage and varied in size from barely perceptible spots to those 3–4 mm. in diameter. Symptoms somewhat similar to these are described by Aderhold[1] for a leaf spot on *Scorzonera hispanica* L. (black salsify) in Germany and by Archer[2] for a leaf spotting of *T. porrifolius* in West Virginia. In both of these cases the disease is caused by *Sporodesmium scorzonerae* Aderh. Morphological studies show that this fungus is entirely different from the one here described.

SUSCEPT RANGE

The only known suscepts are *Tragopogon porrifolius* and *T. pratensis* L. (goat's beard), a fairly common weed in the northeastern United States. Although the disease has not been found on the latter plant under natural conditions, successful inoculations of young, potted plants were made with spore suspensions of the pathogen. The symptoms are similar to those on *T. porrifolius*. Both young and nearly-mature plants of *Scorzonera hispanica* were similarly but unsuccessfully inoculated. In addition, plants of this species were grown in the field in the same rows with cultivated salsify plants. Although the latter eventually became severely blighted, the black salsify plants remained healthy.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The disease seems to be rather limited in distribution, partly because the suscept is not widely grown. A letter was sent out in 1938 requesting diseased material or cultures of the pathogen. Replies were received from pathologists in 19 vegetable-growing states. No reports of any salsify leaf-spot diseases were received, although a plant pathologist in Ohio wrote that he had "seen salsify in one or two instances which was affected with other things than white rust but the trouble never seemed important enough to cause much concern." These replies indicate that salsify is grown on a rather limited commercial scale in 10 States and not at all in 4. The extent of salsify culture in 5 States was not ascertained.

Diseased leaves have been collected each year since 1933 in every commercial planting on Staten Island. The only other collection of which there is a record is that made in 1938 in a small planting near Rochester,

[1] Aderhold, R. Ueber eine bisher nicht beobachtete Krankheit der Schwarzwurzeln. Arb. Biol. Abt. für Land- und Forst. 3: 439–440. 1903.

[2] Archer, W. A. Plant Diseases in West Virginia in 1928. Bur. Pl. Ind., U. S. Dept. Agr. Plant Dis. Rptr., Suppl. 72: 346. 1929.

New York, by A. G. Newhall. The question as to why this disease is so localized is as yet unanswered.

The total area annually devoted to salsify on Staten Island amounts to less than 2 acres and has a value of approximately $1,000. The annual loss from this disease averages approximately 5 per cent of the crop, attributable largely to reduced size and quality of roots. On 2 occasions during the past 6 years, the disease became so severe following prolonged warm, rainy periods that some plants lost all of their foliage. Under enphytotic conditions, which ordinarily prevail, this loss is not more than 30 per cent. Occasionally, soft-rot organisms invade the roots through the severely blighted leaves, the resulting root decay contributing substantially to the economic loss.

SYMPTOMS

The initial symptoms of the disease appears as light-brown lesions on the tips of the older leaves. These ordinarily appear after August 1, when the plants are nearly half-matured. Soon thereafter, minute, light-brown, necrotic spots, surrounded by light-green areas develop on the leaf blades (Fig. 1, A). Later, these lesions enlarge until they are from 3 to 4 mm.

FIG. 1. Symptoms of stemphylium leaf-spot disease of *Tragopogon porrifolius* (cultivated salsify). A, incipient and B, advanced stages of spotting. C. Death of foliage resulting from early-season infections.

in diameter and become cinnamon-brown with ashen-gray centers (Fig. 1, B). A reddish-brown exudate, composed mainly of the natural milky juice of the plant, sometimes can be found on the smaller lesions on both upper and lower leaf surfaces. The larger spots often coalesce and involve con-

siderable areas of the leaves. Severely-affected leaves gradually turn yellow and wither (Fig. 1, C).

THE PATHOGEN

Morphology. The fungus grows readily on potato-dextrose agar and forms in both Petri-dish and test-tube cultures a spreading, cottony growth marked by faintly-zonate bands of conidiophores and conidia. The mycelium is at first light-brown and, finally, a color approaching olive-ocher.[3] The relatively hyaline mycelium ramifies throughout infected leaf tissue, transverse growth in the leaf occurring prior to any considerable amount of lateral growth.

The conidiophores, which are borne in fascicles on the superficial mycelium, are light-brown, occasionally branched, 4.0–4.5 μ wide by 25–180 μ long, and swollen at the apex to a diameter of from 6 to 8 μ (Fig. 2, D, E). The lower two-thirds of the apical swelling is darker than the upper portion and is thickened by an inner wall. Each conidiophore bears a single apical spore. The conidiophore may continue growth (proliferate) through the swollen apex and the scar left by the fallen spore, with a second spore produced terminally. This phenomenon results in the formation of so-called nodular segments, although not more than two of these have been observed on single conidiophores produced either on lesions or in culture.

The spores are light-brown and minutely verrucose, measure 17–56 × 8–26 μ, are ovoid-oblong to subangular, have one to several longitudinal walls and 1–3 transverse walls, and are without appendages or beaks (Fig. 2, C). The spores are slightly constricted at the transverse walls. A basal scar is visible. Occasionally, on germination in culture, the spores give rise directly to conidiophores that proliferate and produce acrogenous spores.

Aderhold's[4] description of the spores of *Sporodesmium scorzonerae* (inverted club-shape and long-drawn-out, measuring without the appendage 50–75 × 13.5–16.5 μ, and with 10–12 cross walls and 1–2 longitudinal walls) leaves no doubt as to the dissimilarity of the two pathogens (Fig. 2, A). Archer (2) identified the causal agent of a leaf spot in West Virginia as morphologically similar to if not identical with *S. scorzonerae.* The spores measured 18–54 × 7.5–8 μ, being somewhat smaller than those described by Aderhold. An examination of spores found on lesions on the diseased material collected by Archer (No. 4147, Path. and Mycol. Coll., Bur. of Pl. Ind., U. S. Dept. of Agr.) lends further proof of the separate identity of the fungus found on Staten Island (Fig. 2, B).

Taxonomy. Cultures of the causal fungus were sent to S. P. Wiltshire of the Imperial Mycological Institute, Kew, England, who identified it as a species of *Stemphylium* (subgenus *Eustemphylium*). According to him[5] it differs from the type species (*Stemphylium botryosum* Wallr.) only

[3] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington) 1912.

[4] See footnote 1.

[5] Personal letter dated April 16, 1940.

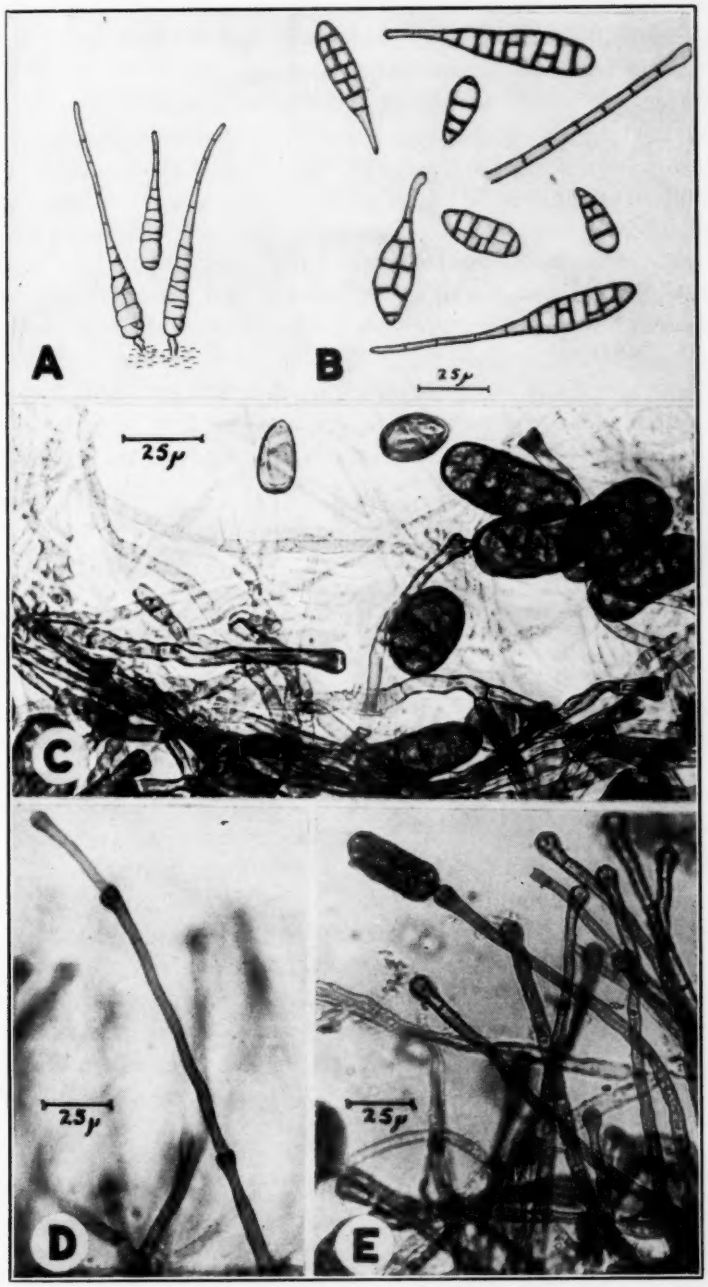

FIG. 2. A. A copy of a drawing by Aderhold of spores of *Sporodesmium scorzonerae* taken from *Scorzonera hispanica* (magnification unknown). B. Camera-lucida drawing of spores of *S. scorzonerae* found on *Tragopogon porrifolius* by Archer. C, D, E. Photomicrographs of spores and sporophores of *Stemphylium botryosum* var. *tragopogoni* from *T. porrifolius*.

in that the spores are longer than is usual for this species (24–39 × 19–31 μ). He was unable to obtain perithecia on exposure of the cultures to light, thus confirming the results of similar experiments performed by the writer. Dr. Wiltshire writes further that in the absence of the perithecial stage "perhaps the least objectionable course would be to regard it as a strain or variety of *S. botryosum* for the time being." Wiltshire[6] has stated that if his conception of the specific limits is correct, the synonyms of *S. botryosum* will probably be comparatively numerous. For example, he considers this name to be synonymous with *Macrosporium sarcinula* Berk. (*M. parasiticum* Thüm.), which has a wide range of suscepts. Under these conditions, it would seem unwise to create a new species on the basis of spore measurements alone, without first making numerous cross inoculations. Therefore, it is proposed that the name *Stemphylium botryosum* Wallr. *Tragopogoni* n. var. be assigned provisionally to this pathogen on *Tragopogon porrifolius*.

Stemphylium botryosum Wallr. **tragopogoni** n. var. Mycelial mat spreading, cottony, faintly zonate, at first light-brown, finally olive-ocher; hyphae septate, branching, light-brown, 3.0–3.5 μ thick; conidiophores arising in fascicles from superficial mycelium, light brown, occasionally branched, swollen at the apex to a diameter of 6–8 μ, proliferating through the apex and fallen spore scar, 4.0–5.5 μ wide by 25–180 μ long; conidia borne acrogenously, light brown, minutely verrucose, 17–56 × 8–26 μ, ovoid-oblong to subangular, 1 to several longitudinal walls and 1–3 transverse walls, constricted at the transverse walls, basal scar visible.

Type material (infected leaves of *Tragopogon porrifolius* and dried agar cultures) has been deposited in the herbarium of the Department of Plant Pathology, Cornell University (Accession No. 25439).

PATHOGENICITY

The pathogenicity of the fungus has been demonstrated repeatedly by inoculating the leaves of potted salsify plants with spore suspensions from single-spore and mass cultures. These plants were then maintained under bell glasses at high humidity and at temperatures of 21–27° C. The first symptoms, consisting of small, water-soaked, irregular areas, became evident within 24 to 36 hours after inoculation. The bell glasses then were removed, and within another 24 hours small, brown spots appeared on these hydrotic areas. If the bell glasses were not removed, conidia were produced on the infected areas within 72 hours after inoculation. Lesions also developed if blocks of agar bearing the mycelium, alone, were placed on the leaves.

EFFECT OF TEMPERATURE ON GROWTH AND SPORE PRODUCTION

The pathogen grows most rapidly and fruits most abundantly at moderately high temperatures. The fungus was grown on 2 per cent potato-dextrose agar (pH 6.5) in triplicated Petri dishes at temperatures ranging from 6° C. to 33° C. The average colonial diameters in millimeters after 10 days were as follows: 6°, no growth; 9°, 17; 12°, 23; 15°, 30; 18°, 44; 21°, 55; 24°, 80; 27°, 52; 30°, 38; 33°, no growth. Spore production was

[6] Wiltshire, S. P. The original and modern conceptions of Stemphylium. Trans. British Mycol. Soc. **21**: 211–239. 1938.

correlated closely with growth: no spores forming either below 15° C. or above 30° C. and the maximum number between 21° and 27° C. Variations in chromogenesis in the media were marked throughout the above temperature series: dark green at 9° C., through yellowish-brown at 24° C. to pink at 30° C.

SEED TRANSMISSION

In order to determine if salsify seed is susceptible to invasion by the pathogen under artificial conditions, and, therefore, a possible agent of transmission, flowers in full bloom in the greenhouse were atomized with spore suspensions of the fungus. The flowers were then enclosed for 36 hours in cellophane bags containing moistened cotton. The bags were then removed and the seed allowed to mature in the flowers. Following harvest the seed was stored in a dry place for 2 months. At the expiration of this period, 100 of these seeds were given individually an instantaneous dip in 95 per cent alcohol, rinsed immediately in water and treated in calcium hypochlorite solution (1:14) for 5 minutes. Following this treatment they were planted on potato-dextrose agar. Cultures of the pathogen were obtained from 80 per cent of the seed. Four months after harvest another lot of this seed was disinfested as above and planted on agar. None yielded cultures of the pathogen. Germination tests made immediately after harvest showed that only 5 per cent of the inoculated seeds were viable, while tests made again at the end of 4 months revealed that by this time none of the seed was viable. Similar tests with seed from noninoculated and unbagged flowers showed that 70 per cent of the seed germinated immediately after harvest and 65 per cent at the end of 4 months after harvest. These results indicate that under certain artificial conditions, not conducive to normal development of the seed, the fungus can penetrate the seed coat and remain alive for relatively short periods of time.

There is no evidence that the pathogen is carried in or on commercial seed despite the demonstrated susceptibility of the seed under abnormal conditions. Fresh seed was obtained from 12 seed companies and from growers on whose farms the disease is enphytotic. Equal representative samples from each lot were either planted in agar or sown in steam-sterilized soil. In some instances the seed was sown without treatment; in others it was disinfested either in calcium hypochlorite solution (1:14) for 5 minutes or $HgCl_2$ solution (1:1000) for 5 minutes followed by two rinses in sterile water. The pathogen was never recovered from the culture media or isolated from the seedlings.

In other experiments, seed from growers' stocks was sown on farms which had not grown salsify for 10 or more years and which were at least $\frac{1}{2}$ mile from the nearest commercial planting. The resulting plants remained healthy throughout the season. If, however, old, blighted leaves were raked into the soil around some of the young seedlings, blight developed on these plants by the latter part of August. When this seed was sown

on farms that had grown salsify year after year, blight appeared on the resulting plants in due course.

It would seem that transmission of the pathogen in or on commercial seed, if it ever occurs, is comparatively unimportant in the life history of the fungus. The fact that the disease appears to be confined almost entirely to Staten Island is a further indication of the minor role played by the seed in the dissemination of the pathogen.

SOIL-BORNE INOCULUM

It has been established through experiment that the fungus is capable of overwintering for at least a year on blighted leaves in the field. Severely-diseased salsify leaves were wrapped in cheesecloth and buried 3 in. below the surface of the soil on Staten Island in late October. Part of the leaves were dug up in July of the following year. One portion was strewn over the leaves of five 6-month-old, potted salsify plants, while a second portion was sterilized in an autoclave before being scattered over a similar series of plants. All plants, including noncontaminated checks, were sprayed with water and placed in an incubation chamber under near optimum conditions for infection. Symptoms of blight developed only on those plants contaminated with nonsterilized, blighted leaves. No stromatic tissue was observed in or on the overwintered leaves although viable spores were found immediately after the leaves were taken from the ground. The remainder of the buried leaves was left in the ground for an additional 12 months. Inoculation experiments, similar to those detailed above, demonstrated that the pathogen was no longer capable of causing infection. Spores could not be found. It is likely that, under some conditions, the longevity of the pathogen in the soil exceeds one year. In any event, the amount and disposition of soil-borne inoculum is believed to be an important factor in conditioning the prevalence and severity of the disease.

CONTROL

Control experiments have been directed mainly toward finding a protective fungicide for the foliage. As a result it has been found that the disease can be controlled readily by spraying with Bordeaux mixture if applications are begun before the older leaves bend over to the ground. Preliminary laboratory and field tests revealed that both 325-mesh sulphur dust and wettable-sulphur sprays were relatively ineffective in preventing spore germination. Copper-lime dust (20–80) was highly toxic to the spores in laboratory tests but did not give satisfactory control in the field because the dew forms in droplets on the waxy-leaf surfaces instead of in a continuous film. Bordeaux mixture (4–4–50) and Cuprocide 54 (Rohm and Haas) ($1\frac{3}{4}$ lb. to 50 gal. water) gave excellent coverage when amended with suitable spreading agents. The best spreaders (1:400) were Pyrolene M.P. (Standard Agricultural Chemicals) and Ultrawet (Atlantic Refining Co.). Others tested were Penetrol (Kay Fries Chemicals), Stantex spreader soap (Standard Agricultural Chemicals), Grasselli sticker and spreader, Grasselli IN–438 (sodium oleyl sulphate), and Santomerse (Monsanto Chemical Co.).

In a series of experiments in 1938 Bordeaux mixture and Cuprocide 54 were applied with a knapsack sprayer, commencing on July 7 when the plants were from 6 to 10 in. tall, and continuing at weekly or biweekly intervals until Oct. 5, 1 week before harvest.[7] Treatments were replicated twice on each of 3 farms with individual plots measuring approximately 10 sq. ft. Data were taken at harvest on the average number of spots per leaf, including both healthy and diseased leaves, on 10 plants chosen at random in each plot. There was no significant difference in this respect between plots receiving weekly applications of either Bordeaux or Cuprocide 54 and those sprayed biweekly. Plants sprayed biweekly with Bordeaux had fewer lesions (1.3 per leaf) than either those sprayed with Cuprocide 54 (6.1) or the nonsprayed checks (11.6). On the basis of root weights correlated with the number of roots required to make a bunch, Bordeaux- and Cuprocide-treated plots yielded approximately 200 more bunches to the acre than did the checks. There was no appreciable yield difference between these two treatments.

SUMMARY

An apparently undescribed leaf spot of cultivated salsify, *Tragopogon porrifolius*, caused by a variety of *Stemphylium botryosum*, occurs commonly in commercial plantings on Staten Island, New York. *T. pratensis* (goat's beard), a common weed, inoculated with the causal fungus, proved susceptible.

The most prominent symptoms are small, light-brown, necrotic spots that cause affected leaves to turn yellow and wither.

The pathogen is morphologically unlike *Sporodesmium scorzonerae*, previously reported on *T. porrifolius* and *Scorzonera hispanica*, and is described herein as *Stemphylium botryosum* Wallr. *tragopogoni*, n. var.

Under optimum conditions of temperature and humidity, inoculation of the suscept results in the formation of lesions within 48 to 60 hours. The optimum temperature for growth and spore production in culture is between 21 and 27° C.

There is some experimental evidence that commercial seed is not an important factor in the overwintering and dissemination of the pathogen.

Soil-borne inoculum, provided by old, blighted leaves from the previous crop, is believed largely responsible for the enphytotic occurrence of the disease.

The disease can be controlled readily through biweekly applications of Bordeaux mixture or Cuprocide 54, provided the spraying program is begun before the older leaves bend over to the ground and provided a suitable spreader is used.

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[7] The common practice on Staten Island is to harvest most of the crop before the first killing frost. The roots are sold in the New York City market in bunches of from 10 to 20 each.

VARIABILITY IN REACTION OF WHEAT DIFFERENTIAL VARIETIES TO PHYSIOLOGIC RACES OF TILLETIA LEVIS AND T. TRITICI[1]

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INTRODUCTION

Experiments previously reported (2) gave evidence that environmental factors may affect the response of certain varieties to some races of the bunt fungi. In those experiments race 2 of *Tilletia levis* Kühn produced 77 per cent infection on Turkey wheat (C.I. 6175) at Pullman, Washington, as contrasted with 16 per cent infection when the same lot of inoculated seed was sown at Kearneysville, West Virginia. Similar differences were obtained with races L–1 and L–4. Five other races of *T. levis*, however, showed no notable differences at the two locations in their reaction on Turkey. The fact that Hybrid 128 (C.I. 4512) gave the same reaction to all of the races tested both at Kearneysville and at Pullman suggested that the environmental effect was expressed only in the reaction of certain varieties to certain races. Obviously, this has a direct bearing on race classification in the bunt fungi. It seemed desirable, therefore, to investigate the problem in detail, particularly with reference to the identification and classification of physiologic races. This paper presents the results of tests that were made to determine the range of variability in the reaction of spring wheat differential varieties to certain races of *T. levis* and *T. tritici* (Bjerk.) Wint.

MATERIALS AND METHODS

Lots of each of the 3 spring wheat differential varieties, Ulka (C.I. 11478), Marquis (C.I. 3641), and Canus (C.I. 11637), were inoculated individually with chlamydospores of each of 7 physiologic races of *Tilletia levis* and of 7 physiologic races of *T. tritici* and then grown at 5 experiment stations in 1937 and 7 stations in 1938. Seed of each variety was grown at Pullman, Washington, in 1936 and used as the stock for succeeding years' tests. Before inoculation it was treated with formaldehyde according to the standard dip method, thoroughly washed with water to remove all traces of formaldehyde, and allowed to dry. Inoculum of each race used was increased on its differential host each year at Pullman, and then stored during the winter at 10° C. Powdered inoculum of each of the races was applied at the rate of 0.5 g. to 100 g. of seed. To insure a uniform spore load sufficient seed of each variety was inoculated in one lot then divided for tests at the several stations. In 1937 and 1938 the inoculated seed was spring-sown

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho, Minnesota, Montana, Oregon, Utah and Washington Agricultural Experiment Stations.

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at the rate of 5 g. to the row in duplicate 5-foot rows at Arlington, Virginia; Bozeman, Montana; Aberdeen, Idaho; and Pullman; and at the same rate in fall sowings at Pendleton, Oregon. Tests at Logan, Utah, and at St. Paul, Minnesota, were added to the list of spring seedings in 1938. The smut percentages shown were based on counts of the total number of healthy and infected heads in each row. Fractional percentages of 0.5 or more were recorded as 1 per cent; those below this fraction were disregarded.

EXPERIMENTAL RESULTS

Races of Tilletia levis

Data on the reaction of Ulka, Marquis, and Canus to 7 races of *Tilletia levis* are recorded in table 1. It will be noted that Ulka, used as a check variety, was highly susceptible to each of the races at all of the stations in both seasons. Furthermore, there were no outsanding differences in the percentages of infection on this variety in different localities nor in the 2 seasons in which the tests were made at any one station. A possible exception is Pullman, Washington, where the average for all races was 74 per cent in 1937 as compared with 97 per cent in 1938. The virulence of all races at this station was, however, uniformly less in 1937 than in 1938; and it seems probable that this difference resulted from the less favorable conditions for infection in 1937.

In contrast to the uniform reaction of Ulka, Marquis was extremely variable in the tests in different localities and to some extent in different years, at least at one station. Thus, in 1937, the average percentage of infection on Marquis for all races, was 7 at Arlington, Virginia, 15 at Pullman, Washington, 39 at Bozeman, Montana, and 57 at Aberdeen, Idaho. Likewise, in 1938, there was a wide range in the general averages of infection in different localities. The average infection with all races was only 6 per cent at St. Paul and 7 per cent at Arlington, but at Aberdeen the average was above 50 per cent. The reaction of Marquis to L–3 illustrates the extreme variability that may occur in the response of this variety to an individual race. In 1937 there was 5 per cent infection at Arlington as compared with 12 per cent at Pullman, 46 per cent at Bozeman, and 64 per cent at Aberdeen. Thus, according to the system of race classification adopted by the writers (2), the above reactions would be classified as resistant, intermediate, or susceptible, depending upon the locality in which the test was made. Where Marquis was tested with the 7 races of *Tilletia levis* for 2 years at the same stations the results were constant at certain stations and variable at others. The differences in infection percentages at Arlington and Aberdeen between 1937 and 1938 were slight. At Pullman, the average infection was 15 per cent in 1937 and 33 per cent in 1938. It seems likely, however, that the difference was due, as in the case of Ulka, to less favorable conditions for infection in 1937. At Bozeman, the seasonal response was very marked. The average infection on Ulka was 87 per cent in both years. On Marquis, however, the average percentage of infection

TABLE 1.—*Infection of Ulka, Marquis and Canus by seven physiologic races of Tilletia levis in different localities in 1937 and 1938*

Race No.	Smut infection at													
	Arlington, Va.		Aberdeen, Ida.		Pullman, Wash.		Bozeman, Mont.		Logan, Utah		St. Paul, Minn.		Pendleton,[a] Oreg.	
	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ulka														
L–1	82	86	97	98	85	97	93	90	.....	95	.....	76	86	93
L–2	76	88	97	98	74	98	89	88	.....	94	.....	68	89	93
L–3	83	91	97	98	77	99	78	89	.....	95	.....	90	95	94
L–4	86	88	96	98	73	97	90	89	.....	94	.....	65	93	83
L–5	94	91	96	95	71	94	89	91	.....	97	.....	66	95	88
L–7	95	86	97	81	65	97	84	80	.....	97	.....	93	60	98
L–8	92	90	98	92	71	99	82	83	.....	92	.....	74	94	97
Av. all races	87	89	97	94	74	97	87	87	.....	95	.....	76	87	92
Marquis														
L–1	10	4	38	53	18	28	30	11	.....	15	.....	2	30	71
L–2	9	15	81	64	38	48	57	32	.....	21	.....	14	61	82
L–3	5	15	64	53	12	40	46	20	.....	50	.....	3	56	79
L–4	11	5	37	51	12	30	37	16	.....	30	.....	7	51	76
L–5	7	17	70	48	8	21	47	17	.....	36	.....	3	37	74
L–7	1	7	34	37	9	18	14	14	.....	25	.....	3	32	67
L–8	5	13	72	48	10	46	40	15	.....	30	.....	8	55	83
Av. all races	7	11	57	51	15	33	39	18	.....	30	.....	6	46	76
Canus														
L–1	0	1	1	4	2	2	3	3	.....	0	.....	1	4	26
L–2	0	0	1	3	1	0	6	0	.....	0	.....	1	13	27
L–3	21	30	59	58	29	46	64	32	.....	44	.....	9	68	81
L–4	0	0	1	0	0	0	2	0	.....	0	.....	1	6	12
L–5	0	5	8	23	1	8	14	7	.....	17	.....	1	10	28
L–7	13	26	67	59	25	30	69	29	.....	31	.....	13	55	84
L–8	27	42	66	60	49	32	77	33	.....	44	.....	10	65	87
Av. all races	9	15	29	30	15	17	33	15	.....	19	.....	5	32	49

[a] Fall-sown.

for all races was 39 in 1937 and only 18 in 1938, and the percentage of infection with some of the races differed widely in the two seasons. On the basis of the uniform infection of Ulka, it seems evident that the variability in the infection of Marquis in the two seasons was due to the effect of environment on the host rather than on the bunt fungi. Furthermore, the environmental effect seemed more marked with certain races than with others. For example, L–7 produced 14 per cent bunt on Marquis in both years, while L–8 produced 40 per cent in 1937 and only 18 per cent in 1938.

Unlike Marquis, which was highly variable in its reaction to all of the races, Canus was constant in its reaction to certain races in different localities. As shown in table 1, the resistance of Canus to L–1, L–2, and L–4 was

maintained in all localities both years. Like Marquis, however, it was variable in its reaction to those races to which it is susceptible. For example, on the basis of the 1938 results with L–3, Canus would be classed as resistant at St. Paul, intermediate at Bozeman and Arlington, and susceptible at Pullman and Aberdeen. Canus was relatively constant in its seasonal reaction at Arlington, Aberdeen, and Pullman. However, in Canus as in Marquis, the average percentages of infection at Bozeman were much higher in 1937 than in 1938.

Races of Tilletia tritici

The tests with races of *Tilletia tritici* appear to corroborate the results obtained with races of *T. levis*. As shown in table 2, Ulka was, in general,

TABLE 2.—*Infection of Ulka, Marquis, and Canus wheats by seven physiologic races of Tilletia tritici in different localities in 1937 and 1938*

Race No.	Smut infection at													
	Arlington, Va.		Aberdeen, Ida.		Pullman, Wash.		Bozeman, Mont.		Logan, Utah		St. Paul, Minn.		Pendleton, Oreg.	
	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938	1937	1938
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ulka														
T–3	82	72	97	84	74	98	94	89	.....	93	.....	68	89	86
T–4	93	81	99	93	81	98	93	91	.....	93	.....	91	87	93
T–5	87	74	98	97	78	96	89	89	.....	84	.....	83	85	96
T–7	87	76	96	97	62	99	91	90	.....	96	.....	63	89	93
T–8	72	70	96	97	56	88	91	87	.....	92	.....	50	85	92
T–9	93	79	93	98	76	96	73	79	.....	88	.....	63	91	95
T–10	6	4	13	13	28	11	3	13	.....	6	.....	2	5	65
Av. all races	74	65	85	83	65	84	76	77	.....	79	.....	60	76	89
Marquis														
T–3	12	7	54	42	19	22	54	17	.....	22	.....	4	25	85
T–4	15	6	84	59	25	30	47	16	.....	27	.....	3	40	76
T–5	5	4	53	49	29	38	44	19	.....	37	.....	2	42	80
T–7	9	5	73	55	11	31	56	21	.....	27	.....	4	35	68
T–8	6	3	70	52	20	30	59	14	.....	14	.....	2	45	84
T–9	4	1	11	10	1	7	9	4	.....	5	.....	1	15	58
T–10	3	5	41	44	7	33	28	10	.....	25	.....	2	33	78
Av. all races	8	4	55	44	16	27	42	14	.....	22	.....	3	34	76
Canus														
T–3	17	14	41	47	39	32	63	20	.....	29	.....	14	27	74
T–4	0	0	2	0	0	0	4	0	.....	0	.....	1	3	17
T–5	16	11	62	47	59	32	58	25	.....	35	.....	8	43	74
T–7	1	1	15	15	4	8	16	4	.....	7	.....	3	4	52
T–8	19	24	60	48	14	40	60	18	.....	30	.....	11	52	77
T–9	1	0	4	1	8	0	3	0	.....	1	.....	0	5	20
T–10	0	6	1	0	1	0	1	0	.....	0	.....	0	3	32
Av. all races	8	8	26	23	18	16	29	10	.....	15	.....	5	20	49

consistent in its reaction to races of *T. tritici* in all of the tests. On the other hand, the reactions of Marquis and Canus were rather variable; and as in the case of *T. levis,* the variability is expressed in the reaction with certain races and not with others. Both Marquis and Canus were fairly consistent in their reaction to the individual races in the 2 years at Arlington, Aberdeen, and Pullman, but at Bozeman the response of these varieties to certain races was not the same both years.

Some of the effects of different races of *T. tritici* are noteworthy. Under certain conditions, upon nearing maturity, Ulka tends to become purplish-brown in the glumes and on part of the neck. When infected with some races, particularly T–10, this pigmentation is greatly intensified. At Aberdeen, Idaho, only 13 per cent of the culms from seed inoculated with T–10 produced smut balls in the head. However, many other heads were observed with the intensified pigmentation and with small shrunken grains, which indicated that the smut mycelium was in the plant but had failed to develop chlamydospores in the ovaries. A similar condition was noted at Bozeman. It thus seems evident that Ulka is more susceptible to race T–10 than is indicated by the percentages of infection shown in table 2.

As previously stated, Canus is resistant to 3 races of *Tilletia levis,* and the resistance was maintained when tested in different localities or years. Similar results were obtained with the *T. tritici* races, T–4, T–9, and T–10 on Canus and with T–9 on Marquis. However, in the races of *T. tritici* to which the 2 varieties are susceptible, the variability was very marked, and at Bozeman results differed in the 2 years. At the other stations the behavior in the 2 years was fairly constant. At Bozeman the average infection for all races was 42 per cent in 1937 as compared with 14 per cent in 1938. Again, it appears that this variability was due to the effect of environment on the host rather than on the fungi, as tests with Ulka indicate that the chlamydospores of all races had an equally high degree of viability in both seasons. Canus was affected in a similar manner, as evidenced by the results involving the races to which that variety is susceptible.

The percentages of infection recorded in tables 1 and 2 are based upon the average of counts in duplicate rows. It seemed desirable, therefore, to check the results with more replications. The three varieties were tested in 1939 with L–2, L–7, T–4, and T–5 in five replications in spring plantings at Arlington, Bozeman, Aberdeen, Pullman, and Logan. These data, recorded in table 3, clearly corroborate the results of previous tests. Ulka was uniformly susceptible to each of the 4 races; but both Marquis and Canus were again variable in their response to certain races and could be classed as resistant, intermediate, or susceptible, depending upon the locality in which the test was made. It will be noted that Canus again maintained a high degree of resistance to races L–2 and T–4 at all stations.

There is evidence that certain spring wheat varieties are less susceptible to bunt when planted in the spring than in the fall. In this connection, when Hope (C.I. 8178) was spring-sown at Bozeman in 1936 and 1937, it

TABLE 3.—*Average infection of Ulka, Marquis, and Canus wheats inoculated with two races of Tilletia levis and two of T. tritici, and tested in five replications in different localities in 1939*

Race No.	Variety	Smut infection at				
		Arlington, Va.	Aberdeen, Ida.	Pullman, Wash.	Bozeman, Mont.	Logan, Utah
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
L–2	Ulka	93.9	94.0	93.3[a]	67.0	87.3
L–7		91.4	92.2	93.4	65.5	87.1
T–4		92.1	92.4	95.7	67.7	93.1
T–5		89.4	91.7	94.4	72.1	85.1
L–2	Marquis	9.7	67.1	77.2[a]	26.2	22.9
L–7		1.5	30.2	41.5	17.0	9.0
T–4		4.7	41.7	62.4	21.1	7.2
T–5		8.4	49.6	56.3	23.0	10.0
L–2	Canus	0.5	0.0	0.0[a]	0.0	0.0
L–7		8.0	44.2	38.0	25.7	13.6
T–4		0.0	1.1	0.2	0.0	0 0
T–5		4.9	36.0	46.0	23.2	14.4

[a] Average of 4 replications.

was highly resistant to each of the races listed in tables 1 and 2. However, when fall-sown at Pendleton in 1938, this variety was completely susceptible to all of the races of *Tilletia levis* as well as to races T–8, T–9, and T–10 of *T. tritici* and was intermediate in reaction to T–3, T–4, T–5 and T–6. These results are in agreement with those reported by Heald and Gaines (1) and Smith (3, 4). In the experiment here reported the wheats were fall-sown at Pendleton, Oregon, in tests otherwise comparable to those from spring seeding at the other stations. The percentages of infection on Ulka at Pendleton with all races except T–10 (tables 1 and 2), were approximately the same as at the spring-wheat stations. There were some noteworthy differences, however, in the smut reactions on Marquis and on Canus. On these two varieties the average percentages of infection in 1938 were higher at Pendleton than in the other tests made from spring seedings. The percentages at Pendleton were lower in 1937 than in 1938, but approximately equal to or higher than those obtained at 3 of the 4 other stations. The important differences between reaction under fall and spring-seeding conditions are expressed with certain races. It has previously been noted that Canus maintained its resistance to several races at all of the spring wheat stations. When tested under fall-sowing conditions at Pendleton, however, the resistance was broken down to some extent, particularly in 1938. The average percentage infection of Canus with L–2 at all the spring-wheat stations in 1938 was 0.7 as compared with 27 at Pendleton. With L–4, the corresponding differences were 0.2 and 12 per cent and with T–7, 6.3 and 52 per cent. The breakdown in resistance of Marquis to race T–9 in 1938 was also marked, the average percentage infection for the spring-wheat stations being 4.7 in contrast to 58 for the tests at Pendleton.

The explanation for the differential response of varieties to individual

races of the bunt fungi under different environmental conditions is largely speculative. Results reported by Woolman (5) and corroborated in preliminary tests by the writers, indicate that the bunt fungi enter the epidermis of seedlings of both susceptible and resistant varieties with equal facility. It appears then that the extent to which these fungi may set up parasitic relationships with the host depends upon the physiologic condition of the protoplasm. This protoplasmic resistance or susceptibility is doubtless governed by definite genetic factors, but the expression of these factors may be modified by environment. Thus it would be expected that certain varieties would differ in response to certain races when grown under different environmental conditions. Inasmuch as Ulka was uniform in its response to each of the races when spring-sown in the different localities, it would appear that the variability in response of Marquis and Canus when grown under different environmental conditions was due primarily to the effect of environment on the host rather than on the fungi. Somewhat less probable explanations are that the variability may have resulted from "varietal screening" of the races of fungi or from differential effect of environment on different biotypes.

SUMMARY AND CONCLUSIONS

Bunt experiments at 7 experiment stations demonstrate that a difference in environmental conditions may affect the response of some spring-wheat varieties to certain races of *Tilletia levis* and *T. tritici*. The effect is not evident, however, in the reaction of some varieties with certain other races. The Ulka variety was constant in its reaction at all stations when inoculated with the races to which it is susceptible. Marquis, on the other hand, was so variable in its response to certain races at different stations, that, according to the race classification used by the writers, this variety would be classed as resistant, intermediate, or susceptible to those races, depending upon the location of the tests. In contrast, this variety maintained a high degree of resistance to race T–9 at all stations where the wheat was sown in the spring. Canus was variable in its response in the different localities to the races to which that variety is susceptible but was constant in reaction to the races to which it is resistant.

The most probable explanation for the differential response of varieties to individual races of the bunt fungi under different environmental conditions is that the expression of genetic factors for protoplasmic resistance in the host is modified differently in the different varieties by environment. That is, the host rather than the fungi showed the primary effect of environment.

The data obtained tend to increase the complexity of race identification and of testing for smut resistance in spring wheats. At two stations relatively high percentages of infection were obtained on the susceptible check variety, Ulka; and some differences seem evident in the pathogenicity of certain races on Marquis and Canus. At three other stations the separation

of the races was more distinct, while at Aberdeen the environmental conditions were nearly optimum for infection and for demonstrating the potentialities of individual races. It is evident from the data obtained that tests for varietal resistance should not be confined to a single area as it is possible for a race to be relatively innocuous under what appears to be optimum conditions for infection and then to assume major importance in another environment.

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A LABORATORY APPARATUS FOR STUDYING SETTLING RATE AND FRACTIONATION OF DUSTS

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(Accepted for publication May 23, 1941)

INTRODUCTION

At the outset of research on dusting it became necessary to design a laboratory apparatus to study settling rate and fractionation of dust clouds. Apparatus previously described[3] (1, 2, 3, 4, 5, 6) were not entirely suited for such studies. Accordingly, an apparatus was designed embodying the following features: a simple method of introducing dust into a settling tower, a "magazine" (charge tube) from which practically instantaneous and complete discharge of dust could be obtained by an air blast, and an exposure chamber at the base of the settling tower by which slides or leaves could be serially exposed to the dust cloud. This apparatus has been found to give consistent results.

DESCRIPTION OF THE APPARATUS

The Settling Tower

The tower was made of cellulose acetate film supported by 3 rings cut from plywood and suitably braced (Figs. 1, 2). The top was sealed by cardboard. The tower is 6 feet high and 10 inches in diameter, the cross sectional area being 78.54 sq. in. A 1-in. projection of the film was left at the bottom, below the last ring, to fit into the top of the exposure chamber. This tower is similar in many respects to that developed at Ohio State University.[3, 4]

When in use the tower is grounded[4] by wetting the film with a 10 per cent calcium chloride solution, placing a strip of tinfoil around the base, and attaching a grounded copper wire to the tinfoil. This eliminates trouble from static electricity.

Exposure Chamber for Making Serial Exposures

This can be made any size, as long as its cross sectional area is larger than that of the tower. The one constructed (Figs. 1, 2) is 18 in. square and 6.5 in. high. A hole is cut in the top just large enough to allow the 1-in. projection of the film to fit snugly.

One in. from the top of the chamber a slot is cut to allow for a shutter; this shutter fits into a slot at the back to hold it in place.

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[3] Anonymous. Application of insecticides to leaves in controlled quantity. Ohio State University, Columbus, Ohio. (Mimeographed paper.)

[4] This tower is described anonymously in a mimeographed paper. This paper was secured through the courtesy of Dr. H. A. Waters, Ohio State University Research Foundation, Columbus, Ohio.

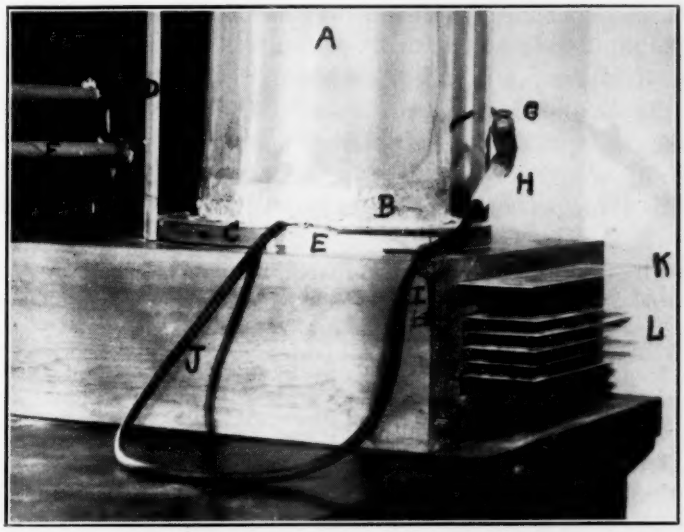

FIG. 1. General view of complete apparatus. A–F, Basal part of tower: A. Cellulose acetate film; B. Tin foil strip; C. Basal plywood ring; D. Plywood strip for support; E. Clamp for holding copper tube inserted in tower; F. Fine copper wire from tin foil to ground on water pipes. G–J. Mechanism for introducing dust into the tower: G. Push button air valve; H. Magazine (charge tube) for holding dust; I. Pinch clamp; J. Rubber tube leading from outlet end of magazine to copper tube inserted in the tower. K–L. Exposure chamber: K. Shutter; L. Aluminum slide holders.

In the front of the chamber, 1.25 in. below the slot for the shutter, a rectangular area, 12.75×3 in., is cut out to allow for the insertion of a drawer (Fig. 2). When in place, this drawer fits into grooves at the sides

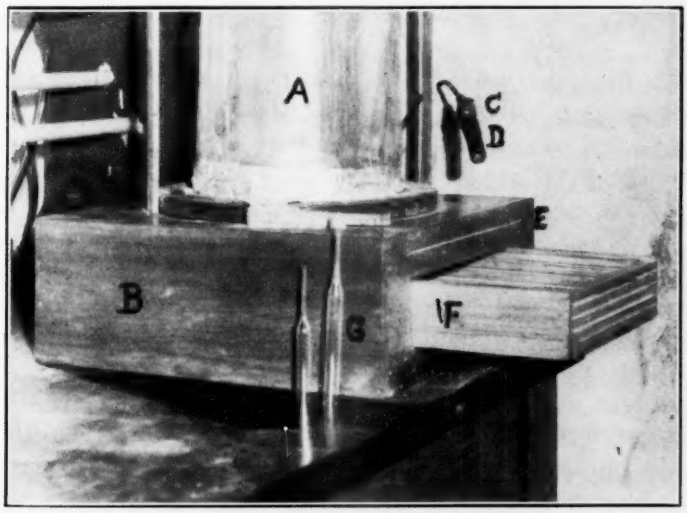

FIG. 2. Detailed view showing certain features. A, Basal part of tower. B. Exposure chamber. C. Push button air valve. D. Large rubber tube from air valve, open end fits over large end of the dust magazine. E. Slot for shutter. F. Drawer fitting into exposure chamber, showing slots for slide holders. G. Dust magazines.

and back of the chamber. The drawer has 5 slots (Figs. 1, 2), each slot being 0.25 in. deep and separated by partitions 0.25 in. thick (Fig. 2).

Sheet aluminum, $\frac{1}{16}$ in. thick, is cut to fit in these slots (Fig. 1) and used to hold the slides or leaves to be dusted. The area of the sheets directly under the center of the tower is marked so that the slides or leaves can be centered.

By this arrangement, 5 exposures can be made at different times to the cloud of dust.

If small potted plants are to be dusted, all that is necessary is to remove the drawer, place the plant in the bottom of the chamber, and close the opening with a piece of cardboard.

Dust Magazine

The dust magazine (charge tube), for instantaneous and uniform discharge of the dust placed in it, should be of certain construction. Two of these are shown in figure 2. For work with relatively large charges, 1 to 3 grams, the tube should be 7 in. long—for 4 inches of its length its inside diameter is $\frac{1}{2}$ in., after which it tapers at one end for a distance of approximately 1 in. until its inside diameter is $\frac{1}{8}$ in. for the last 2 inches. For smaller charges of dust smaller magazines can be used but they should be of the same general construction. The location of this dust magazine is described below.

Various other types of charge tubes were tested and found unsatisfactory. Those that are just large glass tubing with a cork containing a smaller tube at each end, the small tubing at one end connecting with the air supply and the one at the other end being the dust outlet, have been found particularly unsatisfactory for two reasons: not all the dust is discharged and some of it tends to pack at the flat surface of the cork at each end. Those tubes that have the outlet end similar to the "magazine" described above but in which the air enters through a tube in a cork at the other end have been found unsatisfactory also, as the dust tends to swirl back and pack at the corked end. For practically instantaneous and complete discharge of dust from the magazine the tube for the entrance of air should be of the same diameter as the magazine.

Method of Introducing Dust Into the Apparatus

Various methods of introducing dust into the tower were tried. The following method was found to give consistent results.

A copper tube, $\frac{1}{8}$ in. inside diameter, was inserted into the tower at the base through a hole in the cellulose acetate film and held in place by a clothes-pin clamp (Fig. 1). The tube was bent at a right angle in the center of the tower, the vertical extension of the tube being 2 inches. A rubber tube was attached to the end outside the tower (Fig. 1) and connected with the outlet end of the dust magazine. A pinch cock was placed in the line just below the connection. A large rubber tube (Fig. 2), of the same

diameter as the magazine, was fitted over the large (loading) end of the magazine, the other end of the tube being connected to the valve[5] in the air line. This valve operates by a push button.

The entire apparatus, ready for use, is shown in figure 1.

The loading of the magazine is simple. The pinch cock is closed, the rubber tube removed from the large end, the dust poured into the tube, the rubber tube replaced, and the pinch cock opened.

To eject the dust from the magazine into the tower all that is necessary is to push the button in the air valve. All the dust in the magazine is forced out practically instantaneously (less than $\frac{1}{4}$ sec.) by the air blast (15 lb. pressure), and is ejected into the tower in less than one-half second. At 15 lb. pressure most dusts are blown to a height of 5 feet in the tower.

OPERATION AND USES OF THE APPARATUS

The settling rate of a dust is determined as follows: Tarred lantern slides, $4'' \times 3.25''$, are placed on each of the 5 slide holders. A known charge of dust is placed in the dust magazine. If no settling of the aggregates and large particles is desired before exposures are made the top shutter is pulled out, thus exposing the first slide. The dust is then ejected into the tower by opening the air valve for 1 second, timed with a stop watch or metronome. The copper tube is then withdrawn from the center of the tower to avoid interference with the dust cloud. At the 5th second the first slide holder is pulled out, thus exposing the second slide; at the 15th second the second slide holder is pulled out, exposing the 3rd slide; the rest of the slides are handled in similar fashion for the desired time periods. They are then weighed to determine the amount of dust deposited at the various intervals. The time intervals can be varied to suit conditions or the operator.

If it is desired to allow aggregates and large particles to settle before exposures are made, the top shutter is left in while the dust is being ejected and is then pulled out at the desired time, exposing the first slide. Exposures are then made at the desired times as described above.

When fractionation of the component parts of a dust mixture is studied, the procedure is the same as above, except that the deposits are analyzed after weighing to determine the percentage of the various ingredients present at the different time intervals. If more or less of one ingredient is present than calculated at the different exposure periods, fractionation of the mixture has occurred.

Several other types of studies can be made with this same apparatus. These include the deposition of uniform and known amounts of dust on leaves or slides for toxicity studies, deposition of dust on wet and dry surfaces for tenacity studies, and the effect of materials such as oil on settling rate.

In using the apparatus it has been found that the deposit of dust is very

[5] Schrader "Blow Gun" valve, one-fourth inch, No. 7184.

uniform over the exposed area of the slide holder, except for a ring about $\frac{1}{4}$ in. wide at the edge where the deposit is heavier during the first few seconds of settling.

The apparatus should be used in a dimly lighted room, away from sources of light and heat, to obviate convection currents in the tower.

CONSISTENCY OF DATA

The consistency of data obtained with the apparatus is shown by data (Table 1) on the amount of deposit of dust mixtures in a definite time period.

TABLE 1. *Consistency of data obtained with the apparatus, using various dust mixtures*

Dust mixtures[a]	Exposure time[b]	Deposit (13 sq. in.)			
		Rep. 1	Rep. 2	Rep. 3	Average
	Sec.	*G.*	*G.*	*G.*	*G.*
Copper-talc (7–93)	30	.0416	.0421	.0428	.0422
Copper-flour-talc (7–10–83)	30	.0405	.0350	.0380	.0378
Copper-clay (7–93)	30	.0340	.0320	.0350	.0337
Copper-flour-clay (7–10–83)	30	.0340	.0340	.0370	.0350

[a] 1 gram charge of dust used.
[b] 5 second settling period allowed before exposures were made.

A test was made also on the consistency of data obtained for exposure for the first 5 seconds of settling, no presettling being allowed (Table 2). Various diluents were used in this work.

TABLE 2. *Consistency of data obtained with the apparatus, exposing slides for a short period and using various diluents*

Material[a]	Exposure time[b]	Deposit (13 sq. in.)			
		Rep. 1	Rep. 2	Rep. 3	Average
	Sec.	*G.*	*G.*	*G.*	*G.*
Aplite	5	.0490	.0483	.0430	.0468
Cherokee clay	5	.0555	.0565	.0575	.0565
Pyrax ABB	5	.0295	.0310	.0300	.0301
Volclay Bentonite 200M	5	.0383	.0416	.0358	.0386

[a] 1 gram charge of dust used.
[b] No presettling allowed.

The data for the replicates agree surprisingly well in view of the fact that there are two main sources of error in the use of the apparatus: (a) opening the shutter and (b) pulling out the slide holders.

SUMMARY

An apparatus is described for use in studying the settling rate and fractionation of dusts. The features of this apparatus are a settling tower of simple design, an exposure chamber for making serial exposures of slides

or leaves to the dust cloud in the settling tower, a dust magazine (charge tube) from which almost instantaneous and complete discharge can be obtained by an air blast, and a simple mechanism for introducing known charges of dust into the settling tower.

Consistent results have been obtained with this apparatus.

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CEPHALOSPORIUM LEAF SPOT OF DIEFFENBACHIA

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(Accepted for publication May 19, 1941)

INTRODUCTION

Throughout the past 2 years, a leaf spot of *Dieffenbachia picta* Schott (family—Araceae), caused by a species of *Cephalosporium*, was prevalent in greenhouses on Staten Island, New York. The spots were sufficiently large and numerous to make unsalable approximately 400 out of a total of 3,000 plants. A comparison of the symptoms of this disease with those of the bacterial disease of *Dieffenbachia* described by McCulloch and Pirone[1] shows the resemblance between them to be slight. In addition, congo-red tests[2] reveal that bacteria, ordinarily, are not present in the lesions, although colonies of saprophytic bacteria sometimes develop in cultures from lesions and leaf tissue.

SYMPTOMS

The initial infections appear on the young, convolute leaves as tiny, reddish-brown, circular to elongated lesions (Fig. 1, A). As these leaves unroll and expand, the lesions increase in diameter to 6 to 8 mm. At this stage they are mostly circular in outline and rather sharply delimited by a dark-brown border (Fig. 1, B). The central portion is grayish. Elongated lesions are found occasionally on the petioles and midribs, and even on the stems, under conditions extremely favorable for the development of the fungus. Coalescence of the lesions, and yellowing and death of entire leaves are, however, rarely encountered under greenhouse conditions.

PATHOGENICITY

A species of *Cephalosporium* has been isolated repeatedly from the lesions, and infection has been obtained both on young, convolute leaves and on older, fully-expanded ones by atomizing them with spore suspensions from single-spore cultures of the isolates. Symptoms appear on the inoculated plants within 3 to 5 days if they are kept at relatively high humidities and at temperatures of from 21° to 27° C.

Infection may occur through both ventral and dorsal leaf surfaces and more readily through wounds, *e.g.*, needle punctures, than through non-wounded surfaces. Although both young and old leaves are susceptible to infection through wounds, only the convolute or the recently-unrolled leaves are susceptible if the surfaces are not wounded. The majority of the lesions on fully-expanded, mature leaves are initiated when the leaves are in the convolute stage.

[1] McCulloch, Lucia, and P. P. Pirone. Bacterial leaf spot of Dieffenbachia. Phytopath. 29: 956–962. 1939.

[2] Burkholder, W. H. A bacterial leaf spot of geranium. Phytopath. 27: 554–560. 1937.

Observations in the Staten Island greenhouses indicated that the common mealy bug (*Pseudococcus citri* Risso), with which these plants often are infested, was responsible for the infection courts (feeding injuries), since the favorite breeding and feeding areas of these pests on *Dieffenbachias* are under the leaf sheaths surrounding the immature leaves. This hypothesis was tested by the following experiment. Several mealy bugs were collected from English ivy plants, which are not susceptible to the disease, and placed in the uppermost leaf sheaths of ten *Dieffenbachia* plants on which the tips

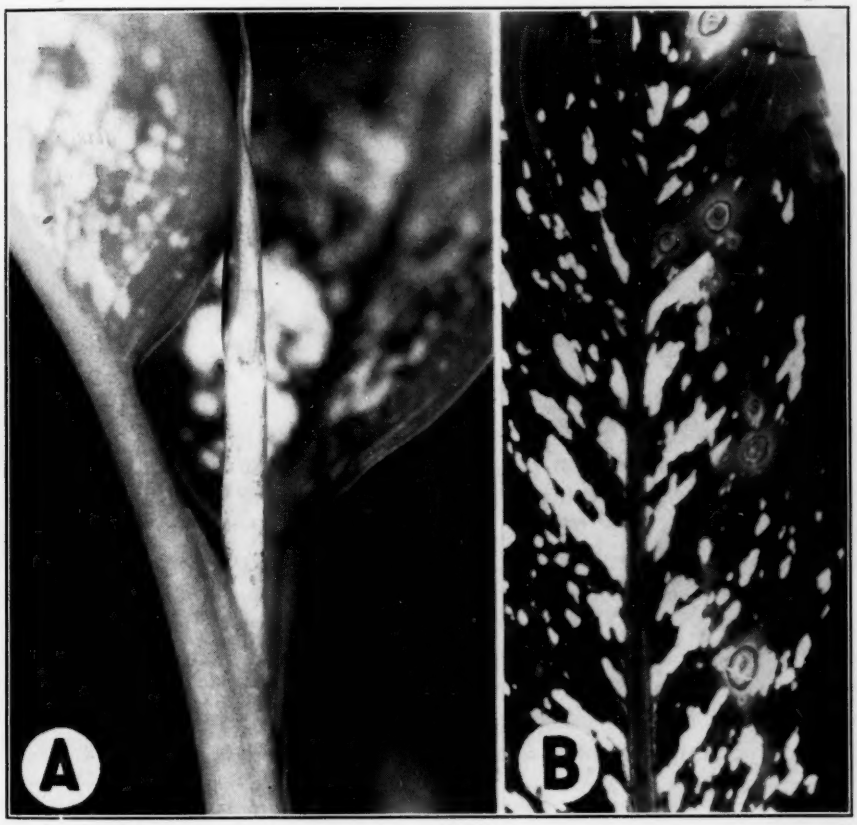

FIG. 1. A. Initial symptoms of *Cephalosporium* leaf spot on young, convolute leaves of *Dieffenbachia picta*. B. Advanced stages of the disease on a mature leaf.

of the new leaves were just beginning to show. At the end of 4 weeks the new leaves were atomized with a spore suspension of the pathogen. A series of noninfested (wound-free) plants were inoculated similarly. A third series of infested and noninfested but noninoculated plants were maintained as controls. Within 5 days numerous lesions had developed on the new leaves with a preponderant number in a more or less linear arrangement above the infested leaf sheaths. Those on the noninfested (and wound-free) plants were considerably less in number and distributed more or less

uniformly over the entire, exposed leaf surfaces. The controls remained healthy.

Cross-inoculation experiments with the *Cephalosporium* from *Dieffenbachia* and with *Cephalosporium cinnamomeum*[3] from *Nephthytis afzelii* Schott, using 20 plants each of *Dieffenbachia* and of *Nephthytis*, demonstrated that the two pathogens are specific in their suscept range and not cross-inoculable.

CULTURAL CHARACTERISTICS OF THE PATHOGEN

On 2 per cent potato-dextrose agar the fungus forms a whitish growth becoming cartridge-buff[4] with age, never cinnamon-buff, as in *C. cinnamomeum*. The mycelial mat is appressed and pulverulent. The branched, septate mycelium gives rise to lateral, upright, simple to 1–2 branched, continuous to septate conidiophores, that, for the most part, are straight and not tapering. Decumbent conidiophores are not produced. This is in contrast to the situation in *C. cinnamomeum*, where the spores from such conidiophores often impart a watery appearance to the surface of the culture. The acrogenous conidia are abstricted singly and collect in grayish-white, slimy, spherical heads. The conidia are ovoid to short-cylindric and have from none to 2, rarely 3 or 4, septa. Chlamydospores have not been observed.

TAXONOMY

The *Cephalosporium* on *Dieffenbachia* differs from *C. cinnamomeum* in (a) suscept range, (b) color in culture, (c) the size of the conidia, (d) the consistency of the mycelial mat, and (e) the shape of the conidiophore. It differs from other species described in the literature principally in suscept range and in color in culture. While there is reason to believe that this fungus may be related closely to some of these species, especially *C. cinnamomeum*, there is no way at present, short of a monographic study of the group, to determine the extent and nature of that relationship. Therefore, it is proposed that the pathogen on *Dieffenbachia picta* be given the name **Cephalosporium dieffenbachiae** sp. nov.

TECHNICAL DESCRIPTION OF THE PATHOGEN

Cephalosporium dieffenbachiae, sp. nov. Mycelial mat creeping, appressed and pulverulent, white, becoming cartridge-buff with age; hyphae septate, branching, 2–4 μ thick; conidiophores arising laterally from the mycelium, aerial, simple to 1–2 branched, continuous to septate, mostly straight, 1.5–2.0 × 4.0–47 μ; conidia borne acrogenously, hyaline, ovoid to short-cylindric, 1.5–2.5 × 3.0–12.5 μ, none to 2, rarely 3 or 4, septa, collecting in grayish-white, slimy, spherical heads measuring 5–12 μ in diameter. On leaves and stems of *Dieffenbachia picta* Schott.

Type material including infected leaves of *D. picta* and dried agar cultures of the pathogen have been deposited in the Herbarium of the Department of Plant Pathology of Cornell University (No. 29413).

[3] Linn, M. B. Cephalosporium leaf spot of two aroids. Phytopath. **30**: 968–972. 1940.

[4] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 col. plates (Washington). 1912.

CONTROL

Although experiments on control have not been attempted, enough is known of the factor influencing the spread and development of the pathogen to warrant some suggestions for control. These include segregation of infected plants, avoidance of syringing, and maintenance of temperatures and humidities as low as is consistent with good cultural practice. Control of mealybugs is highly desirable, but is considerably more difficult of attainment than the other practices.

SUMMARY

The symptoms of a leaf-spot disease of *Dieffenbachia picta*, caused by a species of *Cephalosporium*, are described. The initial infections occur on the young, convolute leaves. The pathogen enters more readily through wounds than through nonwounded surfaces. Most of the wounds are believed to be made by mealybugs feeding under the sheaths surrounding the youngest leaves.

Under experimental conditions, this pathogen and *C. cinnamomeum*, previously reported on *Nephthytis afzelii*, were not cross-inoculable on *Nephthytis* and *Dieffenbachia*.

The cultural and morphological characteristics of the fungus are described in detail.

The name **Cephalosporium dieffenbachiae** sp. nov. is proposed for the pathogen on *D. picta*.

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RELATION OF TUBE LAYERS TO AGE IN SPOROPHORES OF FOMES IGNIARIUS ON ASPEN

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(Accepted for publication May 31, 1941)

It has been assumed quite generally that the age of sporophores of the genus *Fomes* can be determined by counting the number of stratified tube layers in the hymenophore.[2] The writers have found no published studies, however, that demonstrate the accuracy of this assumption. Buller[3] (pages 106–107) cites Faull as having ascertained that *Fomes fomentarius* (L.) Gill., ". . . develops each new tube layer in the autumn." This suggests the probable production of 1 tube layer annually in this species of *Fomes*. White[4] states that, "The sporophores of *Fomes applanatus* are perennial and so a new tube layer is formed during the growing season of each year of its life." He found that the tubes commenced to form by the end of April and their growth was almost complete by the end of July. White, however, does not state whether his observations on tube formation extended beyond a single growing season. Buller[3] indicates (pages 116 and 126) that *Fomes igniarius* (L.) Gill. produces a new annual layer during the first part of the summer, but that it is not known whether each layer of tubes in a fruit body of *F. igniarius* represents the whole of the year's growth. Since a reliable index to the age of perennial sporophores is useful in studying the decay of trees, a project was begun in 1935 to determine the relation of hymenophore stratification to age, using sporophores of *Fomes igniarius* growing on aspen, *Populus tremuloides* Michx.

METHODS

At the Charles Lathrop Pack Demonstration Forest, Warrensburg, New York, 6 aspen trees with 20 sporophores of *Fomes igniarius* on their trunks were selected for study. On August 18, 1935, the sporophores were cut from the trees, care being taken to remove all of the sporophore tissue. The location of each sporophore scar was marked on the trunks with white paint. Each spring, summer, and autumn thereafter the 6 trees were examined and records were made of the season and year when new sporophores appeared on the exposed branch scars. On September 6, 1940, 5 years after the project was started, the new sporophores were removed from the trees and the number of tube layers in each hymenophore was counted.

[1] This project was initiated by the junior author while he was a graduate assistant in the Department of Forest Botany and Pathology at the New York State College of Forestry, Syracuse, New York. Subsequent field observations and records were made by the senior writer.

[2] In this paper, the term "hymenophore" is used to designate that portion of a sporophore consisting of the tube layers, *i.e.*, the trama and the hymenium.

[3] Buller, A. H. R. Researches on fungi. Vol. 2. (London). 1922.

[4] White, J. H. On the biology of *Fomes applanatus* (Pers.) Wallr. Trans. Roy. Canadian Institute. Pages 133–174. 1919.

RESULTS

When the trees were examined on April 4, 1936, the first spring after removal of the original sporophores, a thick layer of newly formed mycelium covered each of the 20 areas previously occupied by the sporophores. At this time 18 of the mycelial formations had shallow pores on their exposed surface, and by summer another of these formations had developed a distinct pore surface; the single remaining mycelial mass never formed pores. In July of the same year a weft of mycelium was found just emerging from a branch scar that previously had not borne a sporophore. By autumn this weft had taken the form of a young sporophore with a pore surface. This sporophore was included in the study; other sporophores that developed subsequently during the 5 years' investigation were not recorded. Thus, there was a total of 20 sporophores of definitely known age.

When the 20 sporophores were removed at the end of the 5-year period, it was found that in 17 of them the age, by summer seasons, corresponded exactly with the number of tube layers (Table 1). In sporophores 3, 14, and 15 the number of tube layers was less than the age in seasons. These 3 sporophores remained small and were almost enclosed by callus at the time they were removed from the trees (Fig. 1, B).

TABLE 1.—*Twenty Fomes igniarius sporophores of known age upon aspen for which the number of tube layers are recorded*

Tree No.	Sporophore No.	Sporophore age in years	No. of tube layers	Sporophore size in cm.[a]	Remarks
1	1	5	5	6.4 × 3.8 × 1.0	
	2	5	5	5.2 × 2.3 × 1.1	
	3	1	0		Callused over 7–3–1937.
2	4	1½	2	4.5 × 4.5 × 0.7	Tree dead, cut down, sporophore removed 7–3–1937.
	5	1½	2	4.0 × 4.5 × 0.7	Same as above.
3	6	2	3[b]	.....	Tree dead, left standing and sporophore removed 6–2–1938.
	7	2	2	.....	Same as above.
	8	2	2	.....	" " " .
	9	2	3[b]	.....	" " " .
	10	2	2	.....	" " " .
4	11	5	5	8.0 × 6.0 × 1.5	
	12	5	5	.....	
5	13	5	5	2.5 × 1.8 × 1.1	
	14	5	2	1.3 × 1.8 × 0.4	Small, crowded by callus.
	15	5	4	1.5 × 0.9 × 0.4	" " " " .
	16	5	5	4.4 × 3.4 × 1.2	
	17	4	4	4.0 × 2.6 × 0.8	Began developing 7–3–1936.
6	18	5	5	5.5 × 2.9 × 1.7	
	19	5	5	5.0 × 3.1 × 2.2	
	20	5	5	5.0 × 2.4 × 1.9	

[a] Measurements of size are in the following order: longest horizontal axis × longest vertical axis × greatest distance from bark to outer margin of sporophore.

[b] The third tube layer was just beginning to form.

The upper surface of the sporophores had concentric ridges (Fig. 1, A)

that corresponded approximately to the layers in the hymenophore, but the ridges were not always sufficiently distinct to be counted accurately. The size of the sporophores also was roughly correlated with the number of tube layers, but varied considerably between sporophores with the same number of layers.

The position of the sporophores on these 6 trees was restricted to branch scars as long as the trees remained alive (Fig. 1). One of the trees (No. 4), however, died in the spring of 1938 and was left standing. By September

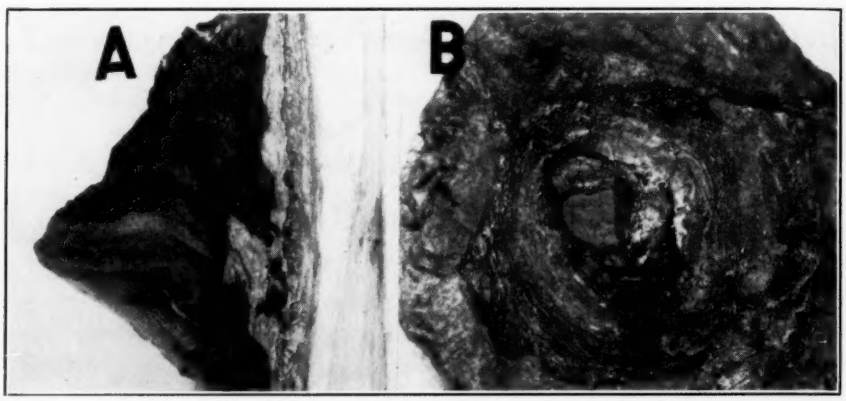

FIG. 1. A. Typical sporophore of *Fomes igniarius* formed at a branch scar on living aspen. This sporophore is 5 years old and contains 5 tube layers. B. Sporophore No. 15, which failed to produce a tube layer annually, possibly because it was almost enclosed by callus. The sporophore has been cut in half for the purpose of counting the tube layers. Both × 1.

of the same year, many new sporophores had formed, irrespective of branch scars, on the trunk of the dead tree where the bark still remained rather firmly in contact with the sapwood. Another tree (No. 2) died and was cut down in the spring of 1937 and left lying on the ground, but no new sporophores formed on this dead trunk.

The results of this study indicate that sporophores of *Fomes igniarius* normally form one tube layer in the hymenophore each year, except when their growth is restricted by surrounding callus or by other adverse factors.

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PHYTOPATHOLOGICAL NOTES

Ring Spot of Apricot.[1]—A destructive disease of apricot (*Prunus armeniaca* L.), characterized by irregular ring spots and blotches in the leaves and fruits, was observed near Palisade, Colorado, in 1935. At that time the condition was noted in a few trees of the Montgamet variety in several orchards. In the following years (1936–1939) the malady was observed to increase in the Montgamet, as well as in the Moorpark, variety.

Leaves exhibited irregular ring spots and a marked clearing of the principal veins. Such manifestations were most apparent on leaves of new shoot growth (Fig. 1, B). As the season progressed, the discolored tissues frequently became necrotic and crumbled away, giving the leaves a frayed and ragged appearance. Developing fruits appeared normal until approximately 2 weeks prior to ripening, about the middle of June. After this time the fruits began to exhibit protuberances, which often gave individual fruits a bumpy appearance. These excrescences were frequently depressed at the apex, giving a cratered effect (Fig. 1, A). The periphery of the papule was frequently discolored, forming a water-soaked green circular margin. As the fruits ripened, this water-soaked green color changed in most cases to a buffy citrine[2] and finally to a red-purple or red-brown hue. During the ripening process the affected fruits filled out, tending to eliminate their bumpy appearance but leaving reddish-brown blotches or ring spots (Fig. 1, C). Ripe fruits with such symptoms often showed cracks in the discolored areas. Sections cut through discolored portions of both green and ripened fruits revealed a necrotic condition of the tissues (Fig. 1, D). Such necroses followed the external form of the ring or blotch. The discolorations usually extended from $\frac{1}{16}$ to $\frac{1}{8}$ in. into the flesh, although necrosis occasionally was found to a depth of $\frac{1}{4}$ in. It has been observed that on any thoroughly diseased limb all the fruits showed such symptoms as those just described. Trees showing an infected limb or twig in one growing season invariably became completely diseased within one or two years. Infected trees have been observed for a period of 5 years, and in no instance have any of these trees showed recovery from the diseased condition. It has been noted that in orchards where diseased trees were eradicated in 1937 and 1938 there was no further spread of ring spot.

However, orchards containing diseased trees that were not removed showed an evident annual increase in the number of diseased trees.

Since repeated tissue plantings from necrotic tissues on nutrient culture media were negative, and the leaf symptoms were suggestive of virus infection, this latter possibility was investigated.

A study was undertaken to ascertain whether the disease could have been caused by the virus of peach mosaic. In the spring of 1936, cions taken from

[1] Paper No. 121 of the Scientific Journal Series of the Colorado Agricultural Experiment Station.

[2] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington.) 1912.

healthy Elberta peach trees were inserted into 5 large, bearing Montgamet apricot trees showing symptoms of the malady. Two peach cions were inserted into each tree. The cions made growth unions but showed no symptoms during the growing seasons of 1936 and 1937. In addition, in the fall of 1936, buds from diseased apricot trees were inserted into 20 Elberta peach nursery trees. In the spring of 1937 shoots from the inserted apricot buds showed leaf symptoms of the malady but no symptoms were observed on the peach growth. In like fashion symptoms were shown by the apricot growth during the seasons of 1938, 1939, and 1940, while none were exhibited by the peach growth.

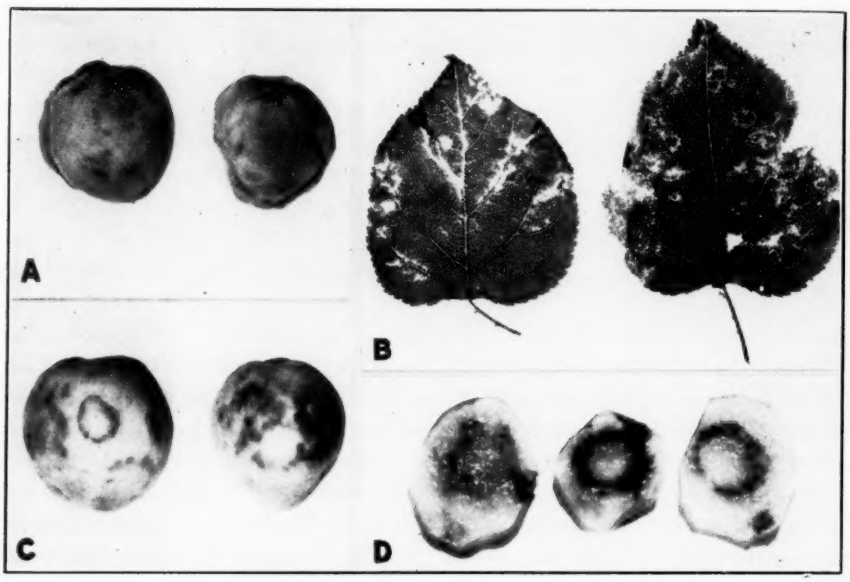

FIG. 1. Ring spot of apricot as expressed on the Montgamet variety. A. Diseased green fruit showing protuberances. B. Leaves from a diseased tree showing vein clearing and ring spots. C. Affected ripe fruit showing ring spots and blotches. D. Sections through diseased fruits showing necrotic areas in the tissue.

Since it was evident by the fall of 1938 that the apricot disease was not caused by the peach-mosaic virus, its possible infectiousness to apricots was investigated. Buds from Montgamet apricot trees showing symptoms of the disease were inserted into 6 healthy Moorpark apricot nursery trees. Two buds were inserted into each tree. The inserted buds made growth unions and showed characteristic symptoms in the spring of 1939 but no symptoms were apparent in the growth of the stock. However, in the spring of 1940 all the stocks produced leaves that showed typical ring spots and vein clearing.

To investigate possible fruit symptoms of the malady, buds from diseased Montgamet apricot trees were inserted into 5 bearing trees 20 to 25 years of age of the same variety in the fall of 1938. Five buds were inserted into

each tree. The majority of the inserted buds made growth unions. During the growing season of 1939 no leaf or fruit symptoms were noted in the growth of the stocks. During the growing season of 1940, however, both leaves and fruit of all 5 trees showed the symptoms of the disease. Ten Montgamet nursery trees inoculated in similar fashion in the fall of 1939 failed to show symptoms in 1940. Both fruit and leaf symptoms, however, were evident on all 10 trees in 1941.

The foregoing studies indicate that ring spot of apricot is of a virus nature in which symptom manifestation is delayed for 2 years. Although there were some similarities in symptom expression, the malady appeared to differ somewhat from the apricot mosaic disease described by Atanasoff[3] as occurring in Bulgaria.—E. W. BODINE and W. A. KREUTZER,[4] Colorado Agricultural Experiment Station, Fort Collins, Colorado.

Wilt Resistance in F_1 Hybrid Watermelons.[1]—That wilt resistance existed in certain citrons (*Citrullus vulgaris* Schrad.) was first shown by Orton.[2] The edible varieties of *Citrullus* in general were wilt-susceptible. Porter and Melhus[3] found that crosses between wilt-resistant types of citron and wilt-susceptible edible melons yielded wilt-susceptible F_1 plants. As a result they emphasized the necessity of growing the F_1 progenies in soil that was not infested with *Fusarium bulbigenum* Cke. and Mass. var. *niveum* (EFS.) Wr. These findings and the fact that segregation of resistance and susceptibility occurred in the F_2 generation led to the assumption that wilt resistance was inherited as a recessive character and that edible melon varieties were homozygous for wilt susceptibility.

In 1937 an inbred selection (inbred 3 generations) from the variety Dixie Queen (wilt-susceptible) was crossed with a wilt-resistant inbred selection from a 3-way cross (Iowa Belle × Jugoslavia 7, backcrossed on Iowa Belle). The purpose of the cross was to develop a round, striped, wilt-resistant watermelon. The F_1 plants of the above cross were 70 to 85 per cent wilt-resistant.

In 1940, 420 hills of the F_1 seed resulting from the above named cross were planted in soil heavily infested with the wilt organism. Three to 6 seeds were planted per hill, but each hill was thinned to a single plant, when the plants were beginning to vine, in order that yield data might be collected. The plants that died subsequent to thinning were killed by the

[3] Atanasoff, D. Mosaic disease of drupaceous fruit trees. Yearbook of the University of Sofia, Faculty of Agriculture 13: 9–42. 1934.

[4] The writers wish to express their appreciation to Dr. L. W. Durrell, Head of the Department of Botany and Plant Pathology of Colorado State College, for helpful advice and criticism.

[1] Journal paper No. J–925 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 71.

[2] Orton, W. A. On the breeding of disease resistant varieties. Inter. Conf. on Plant Breeding and Hybridization, Proc. 1902 (In Hort. Soc. N. Y. Mem. 1: 41–54). 1902.

[3] Porter, D. R., and I. E. Melhus. The pathogenicity of *Fusarium niveum* (EFS.) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad.). Iowa Agr. Exp. Stat. Res. Bull. 149. 1932.

wilt pathogen. By September 1, 1940, 120 plants had been killed and 300 plants, or 71.4 per cent, were alive and bearing full-grown maturing melons.

In another experiment, F_1 seed from 12 additional crosses between wilt-resistant and wilt-susceptible lines was planted in a field heavily infested with the wilt organism. Only 20 hills from each cross were planted, but 2 of the 12 crosses were 80 per cent wilt-resistant. The parentage of these 2 crosses was as follows: Japan 7 × Thurmond Grey; Japan 7 × Dixie Queen. Wilt resistance in the other 10 crosses ranged from 50 per cent to zero, and the 35 hills of the wilt-susceptible Dixie Queen (check) were so susceptible that they all died. The experiment indicated that at least 2 of the hybrids were as wilt-resistant as the F_1 progeny in the previously mentioned experiment.

The fact that some F_1 progenies were wilt-resistant was not in accord with the findings of Orton,[4] who stated that "The first generation should not be exposed to the disease, as resistance may be recessive," and to the work of Porter and Melhus,[5] who found resistance to be a recessive character. In much of the earlier work citrons were used as the primary source of wilt resistance. It is conceivable that wilt resistance was inherited differently in crosses between the citron and watermelon than in crosses involving only watermelon varieties. Regardless of the genetical explanation for the inheritance of wilt resistance it appears clear that in crosses between wilt-susceptible and wilt-resistant edible lines of *Citrullus vulgaris*, the F_1 progenies may or may not be susceptible to *Fusarium bulbigenium* var. *niveum*.—AARON WELCH and I. E. MELHUS, Iowa State College, Ames, Iowa.

The Effect of Chemicals on Fusarium oxysporum cubense Growing in the Soil.[1]—Soil sterilization is practiced in greenhouses and in nursery beds for the control of soil-borne organisms. Brandes[2] used steam in the control of Panama disease and found that it killed out the fungus, but the expense was prohibitive. In order to reduce the spread of Panama disease some of the larger properties in Jamaica used a 1 per cent solution of formaldehyde to treat the banana suckers before they were planted in the field. Sodium nitrate in certain concentrations has been found to reduce the spread of Panama disease.[3] This study has been carried out to determine the per cent of chemical that is necessary to kill *Fusarium oxysporum cubense* in the soil.

The soil cultures used in the experiment were made up with a 0.5 × 8.0 cm. strip of banana leaf, 10 g. of air-dried clay soil, and 3 cc. of water. After the tubes were autoclaved the organism was planted and permitted to grow for 7 days. The chemical was added to soil cultures and allowed to remain

[4] Orton, W. A. On methods of breeding for disease-resistance. Proc. Soc. Hort. Science, 1907, p. 28.

[5] See footnote 3.

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support in this research.

[2] Brandes, E. W. Banana wilt. Phytopath. 9: 339–389. 1919.

[3] Meredith, C. H. The effect of sodium nitrate on *Fusarium oxysporum cubense*. Phytopath. 31: 564. 1941.

for 24 hours before transfers were made to autoclaved banana-leaf tubes for growth observations. Growth could be observed in 24 hours in the checks, but the final reading was made on the third day after transfer.

The percentages given in tables 1 and 2 refer to percentages by weight of chemical to soil. Those of 40 or less in table 1 may be read as percentage of soil and chemical, or percentage of water and chemical. The 50 per cent results were obtained by mixing 1 part of soil culture and 1 part of chemical with 2 parts of water.

TABLE 1.—*The toxic and non-toxic points observed in chemical and soil-culture mixtures*

Chemical	Highest non-toxic *Per cent*	Lowest toxic *Per cent*
98. Ammonium carbonate	6.00	8.00
82. Bismuth nitrate	1.00	5.00
42. Bordeaux mixture	20.00	50.00
4. Boric acid	3.00	4.00
78. Cadmium chloride	20.00	50.00
34. Calcium hydroxide	8.00	9.00
33. Calcium oxide	6.00	8.00
24. Carbolic acid	0.37	0.75
23. Copper spray No. 1	8.00	9.00
39. Copper spray No. 3	30.00	50.00
40. Copper spray No. 4	20.00	50.00
5. Copper sulphate	5.00	6.00
73. Cupric nitrate	7.00	10.00
43. Ethyl mercury iodide	0.0005	0.0025
44. Formaldehyde	0.03	0.04
18. Ferric sulphate	7.00	8.00
49. Ferric nitrate	0.10	1.00
19. Hydrated lime	5.00	6.00
80. Iodine	Saturated solution	50.00
46. Lead acetate	30.00	40.00
35. Lime (air slaked)	10.00	20.00
2. Mercuric chloride	0.04	0.05
45. Mercuric oxide	0.10	1.00
37. Mercurous chloride	0.50	1.00
44. Mercury protonitrate	0.01	0.05
15. Methylated spirits	14.00	15.00
62. Oxalic acid	4.00	6.00
29. Paris green	20.00	50.00
68. Potassium bisulphate	10.00	20.00
70. Potassium chromate	20.00	30.00
69. Potassium cyanide	0.10	1.00
36. Potassium dichromate	0.50	1.00
94. Potassium hydroxide	4.00	6.00
92. Potassium iodide	9.00	10.00
6. Potassium permanganate	3.00	4.00
71. Potassium sulphacyanide	8.00	9.00
76. Potassium sulphocyanate	20.00	50.00
3. Silver nitrate	0.02	0.03
26. Sodium carbonate	20.00	30.00
95. Sodium hydroxide	0.10	1.00
41. Sodium nitrite	0.10	0.50
11. Sodium silicate	12.00	20.00
61. Stannous chloride	4.00	5.00

There was considerable variation in the ability of the chemicals to retard the growth of the transferred spores before the death point was reached.

TABLE 2.—*Chemicals that are not toxic to Fusarium oxysporum cubense in a 50 per cent soil-culture mixture*

Alum	Lead chloride	Potassium ferricyanide
Aluminum silicate	Lead nitrate	Potassium ferrocyanide
Aluminium sulphate	Lead oxalate	Potassium nitrate
Ammonium nitrate	Lead sulphate	Potassium sulphate
Ammonium oxalate	Lead sulphide	Protosulphate
Ammonium sulphate	Litharge	Red lead
Antimony	Magnesium ammonium phosphate	Red iron oxide
Antimony sulphide	Magnesium carbonate	Sodium acetate
Antimony tersulphide	Magnesium oxide	Sodium chloride
Barium chloride	Magnesium silicate	Sodium nitrate
Calcium chloride	Magnesium sulphate	Sodium phosphate
Calcium sulphite	Manganese dioxide	Strontium chloride
Chromium sulphate	Manganese sulphate	Strontium nitrate
Cobaltous chloride	Nickel sulphate	Sugar
Copper spray No. 2	Potassium antimoniate	Sulphur
Iron oxalate	Potassium bicarbonate	Superphosphate
Iron sulphide	Potassium bromide	Zinc carbonate
Indigo	Potassium chlorate	Zinc oxide
Lead arsenate	Potassium chloride	

A 30 per cent mixture of sodium bicarbonate did not kill the fungus. Of the 100 chemicals, 43 killed *Fusarium oxysporum cubense* in the strength mixture given in table 1. In 56 cases the fungus was not killed by a 50 per cent mixture.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Friends College, Highgate, Jamaica, B.W.I.

Growth of Diplodia Macrospora in Media Containing Pure Biotin.—In 1940 Margolin[1] reported that the growth of *Diplodia macrospora* Earle required "biotin or a biotin-like substance." In 1941[2] it was discovered that the substance necessary to a culture medium containing mineral salts and dextrose is produced by the closely related *D. zeae* (Schw.) Lév. As is well known both fungi occur commonly in causal relationship to corn ear rots, although *D. macrospora* is of more limited distribution, being confined largely to warmer regions.

During the past winter it has been found that the addition of pure crystalline biotin-methyl ester[3] to the basal medium supported growth of *Diplodia macrospora*. Some growth was observed with 0.5 γ of the biotin-methyl ester per liter. Much better growth occurred with the addition of 2.0 γ per liter.—NEIL E. STEVENS and R. A. CHAPMAN, University of Illinois.

[1] Margolin, A. S., Proc. W. Va. Acad. Sci., 14 (Keyser), in W. Va. Univ. Bull., Ser. 41, No. 4–11, 1940.

[2] Stevens, Neil E. and W. E. Wilson. Science 93: 458–459, 1941.

[3] Product of the S.M.A. Corp., Chagrin Falls, Ohio.

BOOK REVIEW

OSCAR KRISEN BUROS. *The Second Yearbook of Research and Statistical Methodology.* The Gryphon Press. Highland Park, New Jersey, 1941. pp. XX + 383. $5.00.

The yearbooks of Research and Statistical Methodology, prepared by Prof. Oscar Krisen Buros, are unusual, if not unique, consisting as they do of excerpts of reviews of numerous books appearing in the principal scientific journals. The first yearbook was published under the title "Research and Statistical Methodology Books and Reviews of 1933–38." The second, in part, duplicates the first, "since it is the yearbook policy to continue listing books in successive yearbooks as long as new reviews appear." A total of 1,652 reviews of 359 books or an average of slightly more than 4.5 per book are listed, and excerpts from most of them are included. These reviews were originally published in 283 journals.

Most of the reviewed books deal with statistical methods, in general, or as related to particular fields of inquiry. Economics, education, and psychology are most extensively represented, probably because statistical methods are widely used in these fields and many books explaining their use have been written. Agriculture and biology are represented by a considerable number of books, though relatively few as compared with the above; and chemistry and physics scarcely at all. Several reviews pertain to books dealing with science in general and a few to such miscellaneous subjects as field plot technique, report writing, and general research methods in special fields. The type and printing are excellent. There is included a list of cooperating journals, a periodical directory and index, a publishers' directory and index, an index of names, and a classified index to books.

The need for this book, as seen by the author, arises from the "tremendous advances in statistical theory" that have been made in recent years, the multiplicity of books dealing with statistical methods, and the fact that many of the authors, as well as teachers and investigators, who use these books are "ignorant of recent developments." Many readers will agree with this analysis of the present situation, though probably a good many will hesitate to depend fully on book reviews to indicate with books are least likely to be out-of-date or misleading. Granting that this is an adequate method, it must be admitted that a good job of collecting and excerpting reviews, and arranging them, has been done. Also, no one after reading them will deny that they provide a good starting point for more careful consideration. For the librarian, teacher, or investigator who wishes to add to his collection of books on statistical methods, the present volume should be of much use.

The volume may be useful in other ways, especially for those, if there be such, who have become infatuated with statistical methods. A consideration of the reviews will show beyond doubt that, even though the use of statistical methods will "lead to certainty," as one author has recently stated, it makes a great deal of difference which particular statistical authority one leans upon for advice. Prof. Buros' belief that discrimination is highly desirable appears to be well supported by a recent article by Hotelling to which the interested reader is referred (The Teaching of Statistics, by H. Hotelling. The Annals of Mathematical Statistics. Vol. XI, No. 4, Dec. 1940, pp. 445–470).—S. C. SALMON, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.